

ECOPHYSIOLOGY OF *SPIRODELA POLYRHIZA* (L.)
SCHLEIDEN (GREATER DUCKWEED)



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BY
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RECOMMENDATION

This is to recommend that **Bindu Pokharel (Bhattarai)** has carried out research entitled “**Ecophysiology of the *Spirodela polyrhiza* (L.) Schleiden (Greater Duckweed)**“ for the award of Doctor of Philosophy (Ph.D.) in **Botany** under my supervision. To my knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

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LETTER OF APPROVAL

On the recommendation of Prof. Dr. **Sasinath Jha**, this Ph.D. thesis submitted by **Bindu Pokharel (Bhattarai)**, entitled “**Ecophysiology of the *Spirodela polyrhiza* (L.) Schleiden (Greater Duckweed)**” is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, TU.


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DECLARATION

This thesis entitled “**ECOPHYSIOLOGY OF *SPIRODELA POLYRHIZA* (L.) SCHLEIDEN (GREATER DUCKWEED)**” which is being submitted to the Central Department of Botany, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Sasinath Jha, Department of Botany, Post Graduate Campus, Tribhuvan University, Biratnagar, Nepal.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.



Bindu Pokharel (Bhattarai)

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ABSTRACT

Spirodela polyrhiza (greater duckweed) is the most conspicuous duckweed of the eutrophic lentic water bodies at Biratnagar - a vibrant industrial city of Nepal. To explore the environmental and socio-economic benefits of this hitherto neglected species, the major objectives of the present study were to investigate its growth pattern (in natural habitats as well as on different nutrient media), conditions for turion formation, use as fishmeal replacer in tilapia fish (*Oreochromis niloticus* L.) farming, water cleansing efficiency, response to mercury stress, and use as manure in brinjal and chilli cultivations at Biratnagar.

In an eutrophic pool the highest fresh biomass (823 g/m²) and nutrient concentration (protein 19.6 %, phosphorus 0.9 %, and potassium 2.1 %) in *S. polyrhiza* occurred in winter (January) when concentration of dissolved nitrogen (8.2 mg/l), phosphorus (0.5 mg/l) and potassium (19.7 mg/l) in habitat water were also the highest in comparison to summer (May) and rainy (September) seasons. Further, *S. polyrhiza* fronds exhibited distinct cycle of decrease (senescence) and increase (rejuvenation) in size at intervals of 1 - 4 months in three eutrophic pools of similar water depth and nutrient source (domestic sewage).

When incubated alone *S. polyrhiza* tolerated pH range of water between 4 - 7 with optimum growth at pH 6 but survived in 2 - 10 range of water pH when incubated in mixture with *Lemna aequinoctialis* Welwitsch. The best concentration of inorganic fertilizer per litre borewell water for growth was N 10 mg, P 2 mg and K 2 mg; whereas in comparison to the various concentrations of glucose, sucrose and xylose in borewell water, *S. polyrhiza* incubated on 2 g/l sucrose had the best growth.

At 22nd day of transfer from an eutrophic pool (pH 7.1, N 4.30 mg/l, P 0.06 mg/l, K 15.5 mg/l) into borewell water (pH 6.4, N 0.20 mg/l, P < 0.05 mg/l, K 3.26 mg/l) in December, the number of turions formed per *S. polyrhiza* frond (specific turion yield) was 3.2.

The fresh weight gain of the tilapia fingerlings reared on three different feeds (conventional feed: a mixture of wheat flour, soybean flour and powdered local fishes in 2 : 2 : 1 ratio; duckweed meal : powdered *S. polyrhiza* fronds; and 50 % conventional

feed plus 50 % duckweed meal for 21 days occurred in the order : conventional feed (65 %) > 50 % conventional feed plus 50 % duckweed meal (37 %) > duckweed meal (9 %). The conventional feed and duckweed meal were slightly different from each other in protein and phosphorus but highly different in fat (conventional feed 7.89 %, duckweed meal 1.56 %) contents.

Culture of *S. polyrhiza* fronds on wastewater (collected from a ditch used as dumping site for spoiled vegetables) for 30 days increased duckweed biomass from initial 1 g to 3.5 g and reduced the concentration of nutrients in the wastewater in the order : nitrogen (25 %) > potassium (25 %) > phosphorus (22 %). Further, in response to heavy metal stress, biomass of *S. polyrhiza* increased by 43.8 % (in comparison to 20.2 % increase in control) after incubation for a week on 2 mg/l concentration of mercuric chloride in borewell water. Higher concentrations (> 2 mg/l) of mercuric chloride had detrimental effects on both biomass and photosynthetic pigments of the duckweed.

Semi - liquid manure was prepared by keeping fresh (live) duckweeds (mixture of *S. polyrhiza* and *L. aequinoctialis*) in airtight plastic containers for two months. Seedlings of each test species (brinjal and chilli) were planted in 40 earthen pots of 25 cm diameter and 25 cm depth filled with kitchen garden soil at the rate of one seedling per pot, and each test species was segregated into four sets of ten pots each for manuring as follows: control (C) - that received no manure; and 7-D, 14-D and 21-D that received 500ml manure at 7-day, 14-day and 21-day, intervals respectively. In addition to manuring on specified dates all the pots (including control) received 2 l water at every 4-day intervals until 125 days after seedling planting (DAP), and weeds and pests were controlled till the final harvest (134 DAP) of the test species. The result revealed that the number of leaves, leaf area, number of branches, fruit yield and total dry weight per plant were significantly ($p < 0.01$) much more than control under 7-D and 14-D duckweed manurings in both brinjal and chilli crops; and phosphorus concentration in soil under all the frequencies of manuring was astoundingly huge (65.79 - 119.57 ppm) than control (4.42 - 4.92 ppm) at the time of final harvest of both the test crops.

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LIST OF ACRONYMS AND ABBREVIATIONS

ABA	Abscisic acid
ANOVA	Analysis of variances
APHA	American Public Health Association
BFRI	Bangladesh Fisheries Research Institute
°C	Degree Celsius
CF	Conventional feed
cm	centimeter
DAP	Days after plantation
DMRT	Duncan's Multiple Range Test
DWRP	Duckweed Research Project (Bangladesh)
g	gram
GD	Greater duckweed meal
GoN	Government of Nepal
h	hour
ha	hectare
H ₂ PO ₄ ⁻	Hydrogen Ortho-phosphate
K	Potassium
kg	Kilogram
km	kilometer
l	liter
m	meter
µg	micro-gram
µM	micro-molar
µS	micro-Siemens
mg	milligram
min	minute
ml	millilitre
mm	millimeter
MP	Muriate of potash
msl	mean sea level
N	Nitrogen

nm	nanometer
OD	Optical density
P	Phosphorus
p	probability
pH	<i>pouvoir Hydrogene</i>
ppm	parts per million
rpm	rates per minute
SPSS	Statistical Package for the Social Sciences
sq	square
TKN	total Kjeldahl nitrogen
TSP	Triple super phosphate

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1. BACKGROUND

Nowadays, duckweeds are recognized as a promising new commercial crop because they provide high protein animal feed, organic fertilizer, biofuel ethanol and have great applicability in wastewater purification (Hasan and Chakrabarti, 2009; Jha and Pokharel (Bhattarai), 2015).

Duckweeds are the smallest, fastest growing, and the simplest flowering aquatic plants which float on or just below the surface of nutrient-rich still or slow moving bodies of fresh and slightly brackish waters. They are monocotyledons belonging to the family Lemnaceae (Greek *limne* = lake) although they are also classified as the subfamily Lemnoideae within the family Araceae (Sheh - May *et al.*, 2004). Duckweed consists of five genera: *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*. However, the most commonly available species belong to the three genera *Lemna*, *Spirodela* and *Wolffia* (Plate 1). The species of Lemnaceae are found in all possible combinations with each other and other floating plants.

In Lemnaceae, disseminules are complete fronds or seeds, and their dispersal occurs by water movement or through surface adhesion to waterfowls. Duckweeds are adapted to a wide variety of geographic and climatic zones and are distributed throughout the world except in waterless deserts, permanently frozen polar regions, and extremely wet areas with very high precipitation (Landolt, 2006). Most species are found in moderate climates of tropical and subtropical zones. The appearances of duckweed species not previously seen in areas of Europe have been attributed to rising water temperature throughout the world from global warming (Wolff and Landolt, 1994). Some duckweeds appear to tolerate saline waters but they do not concentrate sodium ions in their growth. The apparent limit for growth appears to be between 0.5 and 2.5% sodium chloride for *Lemna minor* L. (Leng, 1999). Table 1.1 presents the distribution range of the duckweeds found in the South Asia.

These plants are very simple, lacking an obvious stem or leaves. The plant body is a few cells thick thalloid frond without any root (*Wolffia*, *Wolffiella*) or may have one (*Lemna*) or more adventitious roots (*Landoltia*, *Spirodela*) devoid of root hairs. The



(a)



(b)



(c)

Plate 1 Common duckweeds: (a) *Spirodela polyrhiza*, (b) *Lemna aequinoctialis*,
(c) *Wolffia globosa* (with *Spirodela polyrhiza*)

bulk of the frond is composed of chlorenchymatous cells separated by large intercellular spaces that are filled with air and provide buoyancy. Some cells of *Lemna* and *Spirodela* have needle-like raphides composed of calcium oxalate. The vascular bundles in all duckweeds are greatly reduced. The upper epidermis in free-floating duckweeds is highly cutinized and is unwettable. Stomata are on the upper side in all five genera. Anthocyanin pigments similar to that in *Azolla* also form in a number of species of Lemnaceae. Roots of duckweeds are usually short but this depends on species and environmental conditions and vary from a few mm up to 14 cm (Leng, 1999). Roots either stabilize the plant on the water surface or assist the plant to obtain nutrients where these are in dilute concentrations. They tend to lengthen as mineral nutrients in water are exhausted.

Duckweeds multiply principally through vegetative propagation by the formation of daughter fronds. New or daughter fronds are produced alternatively from two budding pouches on each side of the mother frond in *Spirodela* and *Lemna*. In *Wolffia* and *Wolffiella* only one budding pouch exists. These budding pouches are situated in *Spirodela* or *Lemna* close to where the root arise, whereas in *Wolffia* and *Wolffiella* the solitary budding pouch is located on the narrow end of the mother frond. Newly formed fronds remain attached to the mother frond during the initial growth phase and the plants therefore appear to consist of several fronds. An individual frond may produce as many as 20 daughter fronds during its lifetime, which lasts for a period of 10 days to several weeks (Landolt, 1986). Generally, however, after six deliveries of daughter fronds, the mother frond tends to die. Duckweed colonies produced in laboratory or naturally are always spotted with brown dead mother fronds. The daughter frond repeats the history of its mother frond.

Vegetative growth in *Lemna minor* exhibits cycles of senescence and rejuvenation, mediated by chemicals released by the mother frond, under constant nutrient availability and consistent climatic conditions (Ashbey and Wangermann, 1949). Fronds of *Lemna* have a definite life span, during which, a set number of daughter fronds are produced; each of these daughter fronds is of smaller mass than the one preceding it and its life span is reduced. The size reduction is due to a change in cell numbers. Late daughter fronds also produce fewer daughters than early daughters. At the same time as a senescence cycle is occurring an apparent rejuvenation cycle, in

which the short lived daughter fronds (with half the life span of the early daughters) produce first daughter fronds that are larger than themselves and their daughter fronds are also larger, and this continues until the largest size is produced and senescence starts again. The cyclic nature of a synchronized duckweed mat (all fronds of the same age) could be over at least one month as the life span of fronds from early to late daughters can be 33 or 19 days, respectively with a three-fold difference in frond rate production (Wangermann and Ashbey, 1950).

When the aquatic ecosystem dries out or declining temperatures occur, duckweeds have mechanisms to persist until conditions return that can support growth. This occurs through late summer flowering, or the production of turions.

Although sexual reproduction is rare in duckweeds, some species, however, reproduce by producing very small unisexual and monoecious flowers and seeds in an inflorescence. In fact, the flower of the duckweed genus *Wolffia* is the smallest known, measuring merely 0.3 mm long (Landolt, 1986). The inflorescence generally consists of one female (pistil) and two male flowers (stamens), but in *Wolffia*, there is one male and one female. The flowers are naked or surrounded by spathe. The fruit is a utricle and the seeds are smooth or ribbed. The seeds are resistant to prolonged desiccation and quickly germinate in favourable conditions.

During the season of short photoperiods or cold nights, the newly developing fronds of several species of duckweed get transformed into small brown to olive green orbicular to reniform dormant bodies, called turions. In comparison to normal fronds, turions have shrunken vacuoles, smaller intercellular space, and abundant starch granules. Because the volume of intercellular space shrinks and starch increases the density of the tissue, the turion can sink to the bottom of the water body where it can survive even if top of water freezes. Turions sprout under the favourable conditions using the stored starch as an energy source to give rise to normal fronds capable of further multiplication. Several species survive at low temperatures without forming turions. During the winter season, their fronds are greatly reduced but remain at the water surface.

Table 1.1 Duckweeds of the South Asia [Cook, (1996)]

Species	FronD	Root	Seed	Habitat and distribution
<i>Landoltia punctata</i> (G. F. W. Meyer) Thompson	Flattened (leaf like), 1.5-8 mm long, 1-5 mm wide, floating	2 - 7	0.8 – 1mm long	A native from the Southern hemisphere and East Asia; naturalized in warm region of Ind (DL, MH, MN, WB)
<i>Lemna aequinoctialis</i> Welwitsch	Flattened (leaf-like), 1 – 6.5 mm long, 0.8 – 4.5 mm wide, floating	Solitary, up to 3.5 cm long, root cap sharply pointed	One per fruit, 0.45 - 0.8 mm long	Eutrophic water bodies like roadside pools, ditches, village ponds, paddy fields, sluggish canals, etc.; often grow together with <i>Spirodela</i> and/or <i>Wolffia</i> ; Pak, Nep, Ban, Mya, SL, Ind (AN, AP, AS, BH, DL, GJ, HP, JH, JK, KL, KT, MH, PJ, RJ, UK, UP, WB)
<i>Lemna gibba</i> L.	Flattened, 4 mm thick, 1-8 mm long, 0.8 – 6 mm wide, floating	Solitary, >3 mm long, root cap rounded	1 - 5 per fruit, 0.7 - 0.9 mm long	Mesotrophic to eutrophic water; Pak, Ind (GJ, JK, PJ)
<i>Lemna minor</i> L.	Flattened, 1-8 mm long, 0.6 - 5 mm wide, floating	Solitary, >3 mm long, root cap rounded	One per fruit, 0.7 - 1 mm long	Mesotrophic to eutrophic water; cooler regions of Pak, Nep, Ind (HP, JK, SK, UK)
<i>Lemna tenera</i> S. Kurz	Flattened, 3.5 - 9 mm long, 1.2 - 3 mm wide, submerged	Solitary, 2.5 mm long, root cap rounded	-	Humid warm region of Mya
* <i>Lemna trisulca</i> L.	Flattened, 3 -15 mm long, 1 - 5 mm wide, submerged except when flowering-fruitlet	Solitary, 2.5 mm long, root cap pointed	One per fruit, 0.6 - 1 mm long	Mesotrophic cooler water (sheltered between emergent reeds); Pak, Ban, Ind (JK, MN, RJ, UK, WB)
* <i>Lemna turionifera</i> E. Landolt	Flattened, 2 mm thick, 0.8 - 3.5 mm long, 0.8 - 3.5 mm wide, floating	Solitary, > 3 cm long, root cap rounded	One per fruit, 0.5 - 0.8 mm long	Temperate regions; Ind (HP, JK)
<i>Spirodela intermedia</i> W. Koch	-	2 - 5	-	Native of tropical and subtropical South America; introduced in Ind (DL)

Contd.....

Species	FronD	Root	Seed	Habitat and distribution
* <i>Spirodela polyrhiza</i> (L.) Schleiden	Flattened, 1.5 - 10 mm long, 1.5 - 8 mm wide, floating	7 - 21	0.7 - 1 mm long	Similar to <i>Lemna aequinoctialis</i>
<i>Wolffia angustata</i> E. Landolt	Boat-shaped, 0.5 - 0.8 mm long, 0.2 - 0.4 mm wide, submerged except when flowering - fruiting	-	0.3 - 0.4 mm long	Eutrophic village ponds; Ind (WB)
<i>Wolffia arrhiza</i> (L.) Horkel (ex WIMM.)	Spherical to ellipsoid, 0.5 - 1.5 mm long, 0.4 - 1.2 mm wide, submerged except when flowering -fruiting	-	0.4 - 0.5 mm long	Cooler water; Ind (JK)
<i>Wolffia globosa</i> (Roxb.) den Hartog et van der Plas	Ellipsoidal, 0.4 - 0.8 mm long, 0.3 - 0.5 mm wide, submerged	-	-	Warm regions; Pak, Nep, Ban, Mya, SL, Ind (AP, AS, BH, CG, DL, GJ, KL, MP, PJ, RJ, TN, UK, UP, WB)
<i>Wolffia microscopica</i> (Griff.) S. Kurz	Upper surface sub-orbicular and lower surface tapering downwards, 0.4 - 1 mm long, 0.3 - 0.8 mm wide, submerged except when flowering- fruiting	-	0.3 mm long	Endemic to warmer regions; Pak, Ban, Ind (AP, DL, GJ, HR, TN, UP)
<i>Wolffia neglecta</i> E. Landolt	Boat- shaped, 0.6 - 0.9 mm long, 0.4 - 0.6 mm wide, submerged	-	-	Eutrophic water; endemic to Pak, SL, Ind (RJ, WB)
<i>Wolffiella hyalina</i> (Delile) Monod	Boat-shaped, 1 - 3 mm long, 0.8 - 2 mm wide, submerged except when flowering- fruiting	-	0.3 - 0.4 mm long	Native of drier regions of Africa; introduced in Ind (Hyd)

* = Turion forming species; Pak = Pakistan, Nep = Nepal, Ban = Bangladesh, Mya = Myanmar, SL = Sri Lanka, Ind = India : AN = Andaman and Nicobar, AP = Andhra Pradesh, AS = Assam, BH = Bihar, CJ = Chhattisgarh, DL = Delhi, GJ = Gujarat, HP = Himachal Pradesh, HR = Haryana, Hya = Hyderabad, JH = Jharkhand, JK = Jammu and Kashmir, KL = Kerala, KT = Karnataka, MH = Maharashtra, MN = Manipur, MP = Madhya Pradesh, PJ = Punjab, RJ = Rajasthan, SK = Sikkim, TN = Tamil Nadu, UK = Uttarakhand, UP = Uttar Pradesh, WB = West Bengal.

1.2. RATIONALE OF THE PRESENT STUDY

Duckweeds, the tiny aquatic plants, have a leaf – like body (composed of soft tissues), called frond that performs photosynthesis. Fronds grow vegetatively and can increase biomass rapidly, lowering carbon dioxide in the air and reducing nitrogen and phosphorus in the water. Duckweeds have been fed to animals and fish to complement diets, largely to provide a protein of high biological value. Besides, the dormant phase of some duckweeds, called turions, is rich in starch - a suitable substrate for ethanol production. However, despite their immense economic and environmental values duckweeds remain unutilized in Nepal mainly due to lack of research and popularization activities. Hence, the present study was designed to explore the growth potentials (both *in-situ* and *ex-situ*) and some economic aspects of *S. polyrhiza* - the most ubiquitous and robust duckweed found at Biratnagar, Nepal.

1.3. HYPOTHESES AND OBJECTIVES

The major hypotheses of the present study were:

- Growth and nutritive value of *Spirodela polyrhiza* depend on physico-chemical properties of water ;
- Decreasing temperature and low nutrient concentration in water induce turion formation in *S. polyrhiza* ;
- *S. polyrhiza* can be used as a fishmeal replacer in farming Nile tilapia (*Oreochromis niloticus*L.) fish ;
- *S. polyrhiza* is capable to cleanse wastewaters contaminated with plant nutrients and heavy metals ;
- The duckweed manure can be used as a reliable fertilizer and soil improver for cultivating vegetable crops (brinjal and chilli).

According to the hypotheses, objectives of the present study were:

- To record variations in morphology, biomass and nutrients in *S. polyrhiza* along with variations in physico-chemical properties of water in selected pools at Biratnagar ;

- To document the growth responses of *S. polyrhiza* on different water pH, and varying concentrations of inorganic fertilizer, and sugars (glucose, sucrose and xylose) ;
- To investigate the effects of decreasing temperature (as in December) and low nutrient concentration in water on turion formation in *S. polyrhiza* ;
- To test the efficacy of *S. polyrhiza* powder as a replacer of fishmeal in finfish (tilapia) farming ;
- To record nutrient removal efficiency and tolerance to mercuric chloride stress of *S. polyrhiza* ;
- To investigate the effects of duckweed manuring on growth and fruit yield of brinjal and chilli crops together with improvement in physico-chemical properties of soil.

CHAPTER 2
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. ENVIRONMENTAL REQUIREMENTS

A variety of environmental factors, such as water temperature, pH and nutrient concentration control the growth and survivability of duckweeds. The other environmental factors that influence the growth rates of duckweed colonies are crowding by overgrowth of the colony, competition from other plants for light and nutrients, and presence of heavy metals in water.

Water temperature: Optimum temperature for maximum growth of duckweeds lies between 16 and 30 °C (Culley *et al.*, 1981; Gaigher and Short, 1986). In Bangladesh, Khondker *et al.* (1993 a) reported the temperature dependent growth of *Spirodela polyrhiza* with a maximum biomass of 76.4 g/m² recorded in the middle of February, after which the biomass depleted gradually with the rise in water temperature. The water temperature in the middle of February was 19°C. Islam and Khondker (1991) also obtained a high growth of *S. polyrhiza* at temperature 16°C. Later, Khondker *et al.* (1993 b) reported maximum growth of *S. polyrhiza* at water temperature of 22.2-22.5 °C in a growth study conducted in pond water. Khondker *et al.* (1994) reported an inverse correlation between water temperature and biomass of *Lemna perpusilla* when the water temperature varied between 15 and 28 °C. These authors also noted that the growth of this duckweed species ceased completely at 26 °C and above.

Water pH: In Bangladesh, *S. polyrhiza* has been reported to grow best at a pH between 6.5 and 7.5 (Islam and Khondker 1991). The range of pH for optimum growth of this duckweed reported from India is 6.8-8.5 (Kaul and Bakaya, 1976; Gopal and Chamanlal, 1991). Khondker *et al.* (1994) reported the pH range of 6.9 and 7.8 to be suitable for the growth of *Lemna perpusilla*, whereas Islam and Paul (1977) reported the best growth of *Wolffia arrhiza* at the pH range of 7-8. Stephenson *et al.* (1980) noted that duckweeds display optimum growth in medium of pH 5-7 whereas, a doubling of biomass in 2 to 4 days has been demonstrated at pH levels between 7 and 8 (Culley *et al.*, 1981). In general, ionized ammonia is the preferred nitrogen substrate for duckweeds. An alkaline pH shifts the ammonium-ammonia balance towards the unionized state and results in the liberation of free ammonia, which is toxic to duckweed at higher concentrations (100 mg/l; Hasan and Chakrabarti, 2009).

Conductivity: Electrolyte conductivity gives an account of accumulation of salts (primarily chlorides and sulphates of calcium, magnesium, sodium and potassium) in water. Upadhyaya *et al.* (2011) reported electrolyte range of 24.8 - 184 $\mu\text{S}/\text{cm}$ for the occurrence of *Lemna aequinoctialis* and *S. polyrhiza* in roadside pools at Biratnagar, Nepal. Zutshi and Vass (1973) found *Lemna gibba* and *L. minor* growing in stagnant water rich in electrolyte ranging from 400 - 500 $\mu\text{S}/\text{cm}$. Gopal and Chamanlal (1991) reported the maximum biomass of *Lemna perpusilla* and *S. polyrhiza* from roadside pools and ditches with a electrolyte conductivity range of 650 - 1000 $\mu\text{S}/\text{cm}$. Khondker *et al.* (1993a) recorded the complete disappearance of *S. polyrhiza* by the end of May when a sharp fall in conductivity and alkalinity was observed. The electrolyte conductivity of water supporting the growth of *L. perpusilla* in Bangladesh reported by Islam and Khondker (1991), and Khondker *et al.* (1994) were 625 and 200 – 890 $\mu\text{S}/\text{cm}$, respectively. High electrolyte conductivity (1090 $\mu\text{S}/\text{cm}$) of water supporting the growth of *L. perpusilla* was also reported by Van der Does and Klink (1991) in a lemnid habitat in the Netherlands.

Nutrients: One of the most important factors influencing the distribution of aquatic plants is nutrient availability (Hutchinson, 1975). Edwards *et al.* (1992) observed that the pond water with less than 3 mg/l total Kjeldahl nitrogen (TKN) and 0.3 mg/l total phosphorus did not support normal growth of *L. perpusilla*. The reason for the comparatively high nutrient demand of free-floating duckweeds resides in the fact that nutrients are absorbed by the lower surface of the fronds which are rather small compared to that of the root hairs of other plants (Landolt and Kandeler, 1987).

The value of duckweed as a feed resource for domestic animals depends on its crude protein content which seems to increase to a maximum of 40 % dry matter over the range from trace ammonia concentrations to 7 - 12 mg/l (Leng *et al.*, 1994). Culley *et al.* (1981) reported that the TKN of water should not drop below 20 - 30 mg/l if the optimum production and a high crude protein content of duckweed are to be maintained. A useful indicator of whether conditions in the pond are appropriate for growth of duckweed (particularly *Lemna* spp.) of high protein content is the length of the roots as there is a close negative relationship between root length and protein content of the duckweed and with the N content of the water. By monitoring this

characteristics, the user can have an indication of nutritive corrective measures when the length of the roots exceeds about 10 mm (Leng, 1999).

Phosphorus is essential for rapid growth and is a major limiting nutrient after nitrogen, although its quantitative requirements for maximum growth is generally low. Fast growing duckweed in nutrient rich water is a highly efficient sink for both phosphorus and potassium; little of each, however, is required for rapid growth. Duckweeds appear to concentrate P up to about 1.5 % of their dry weight and as such are able to grow on high P waters provided the N concentration are maintained. The plants also appear to be able to draw on the pool of P in its biomass for its biochemical activities and once P had been accumulated it will continue to grow on water devoid of P. Saturation of phosphate uptake by duckweed occurs at available PO₄-P concentrations of 4 - 8 mg/l. Rejmankova (1975) reported good growth of duckweed within the P concentration of 6 to 154 mg/l. Culley et al. (1978), working in dairy waste lagoons, achieved doubled production from 2 - 4 days at P concentrations in excess of 35 mg/l. Reduced growth in some species occurs only when P values drop below 0.017 mg/l (Luond, 1980).

Although vigorously growing duckweed is a highly efficient potassium sink, only low concentrations of K in water are needed to support good growth when other mineral requirements are satisfied. Most decaying plant materials would easily produce the K requirements of duckweed.

Most research on nutrient requirements have centered on the need of N, P and K. However, like all plants, duckweeds need an array of trace elements and have well developed mechanisms for concentrating these from dilute sources. From the experience of the PRISM in Bangladesh, it appears that providing trace minerals through the application of crude salt (9 kg/ha/day) is sufficient to ensure good growth rates of duckweeds in ponded systems (Leng, 1999).

Maximum, minimum and optimum requirements of some of the most important environmental parameters (temperature, pH, conductivity, nitrogen and phosphorus) are given in Table 2.1. It is apparent that the duckweeds are robust in terms of survival, but sensitive in terms of thriving (Hasan and Chakrabarti, 2009). They have

extreme range of tolerance for temperature, pH, conductivity, nitrogen and phosphorus with well-defined range of optimum requirement.

Sugars: Like all photosynthetic organisms, duckweeds grow with only requirements for minerals, utilizing solar energy to synthesize biomass. They have, however, the capacity to utilize pre-formed organic materials, particularly sugars, and can grow without sunlight when provided with such energy substrates (Leng, 1999).

Table 2.1 Summary of some important environmental requirements of duckweed (Hasan and Chakrabarti 2009)

Environmental parameters	Minimum	Maximum	Optimum
Temperature °C	0	35	15 - 30
pH	3	10	6.5 - 8
Conductivity (μ S/cm)	200	1090	-
Nitrogen (mg/l NH ₃ -N)	Trace	375	7-12
Phosphorus (mg/l PO ₄ -P)	0.017	154	4 - 8

Table 2.2 Daily fertilizer application matrix for duckweed cultivation (Skillicorn *et al.*, 1993)

Fertilizer Application (kg/ ha)	Daily production of fresh plants (kg/ ha)					
	500	600	700	800	900	1000
Urea	10.0	12.0	14.0	16.0	18.0	20.0
TSP	2.0	2.4	2.8	3.2	3.6	4.0
MP	2.0	2.4	2.8	3.2	3.6	4.0
Sea salt	4.5	5.4	6.3	7.2	8.1	9.0

2.2. AQUACULTURE

Rainwater collected in ponds may need a balanced NPK application which can be given as inorganic fertilizer or as rotting biomass, manure or polluted water from agriculture, sewage or industry.

Urea is a suitable fertilizer, containing approximately 45 % nitrogen, and is rapidly converted to ammonia under normal conditions. Muriate of potash (MP) and triple superphosphate (TSP) each in ratio to urea of 1:5 work satisfactorily as source of potassium and phosphorus, whereas crude sea salt is used as the source of trace minerals (Skillicorn *et al.*, 1993). A fertilizer application matrix aimed to achieve variable daily production ranging from 500 -1000 kg of fresh duckweed per ha was developed by PRISM in their experimental programme at Mirzapur, Bangladesh (Table 2.2). Further, the PRISM recommended daily fertilization rates for different types of duckweed (Table 2.3). The application rate varies from 21 - 28 kg/ha/day (amounting to > 7 tonnes/ha/day) with an anticipated fresh biomass yield of 900 - 1000 kg/ha/day. The daily fertilization rate for duckweed cultivation developed by the Bangladesh Fisheries Research Institute (BFRI) is presented in Table 2.4. The fertilizer schedules developed by PRISM and BFRI are very similar (Tables 2.3 and 2.4), except that BFRI recommended half the dosage of inorganic fertilizer when cowdung was used at the rate of 750 kg/ha/day.

Any waste organic material that is readily biodegradable and has a significantly high nutrient content (Table 2.5) could be used for duckweed cultivation. The most economical sources of such waste materials are all kinds of animal manure, kitchen waste, wastes from a wide range of food processing plants, biogas effluents, and slaughter house wastes. Solid materials such as manure from livestock, night soil from village, or food processing wastes, can also be mixed with water and added to ponds at suitable levels. All wastewater containing manure or night soil must undergo an initial treatment by holding it for a few days in an anaerobic pond, before using it to cultivate duckweed.

Sutton and Ornes (1975) and Said *et al.* (1979) demonstrated the necessity of periodic additions of nutrients to small duckweed culture systems receiving municipal or dairy

cattle wastes. Within 1-3 weeks, there was noticeable drop in N, P and K within the plants. There was a corresponding drop in crude protein as the plant nitrogen declined. In fact, due to high nitrogen requirement of duckweed and the relative rapid loss of nitrogen from aquatic system, this nutrient tend to be limiting in ponds fed with wastewater (Gaigher and Short, 1986). Large scale duckweed production therefore requires the availability of relatively large quantities of organic waste.

Table 2.3 Rates of fertilizer application for duckweed cultivation (DWRP, 1998)

Duckweed	Rate of application (kg/ ha/ day)		
	Urea	TSP	MP
<i>Spirodela</i>	20	4	4
<i>Wolffia</i>	15	3	4
<i>Lemna</i>	15	3	3

Table 2.4 Rates of fertilizer application for duckweed cultivation (BFRI, 1997)

Fertilizer combination	Rate of application (kg/ ha/ day)			
	Urea	TSP	MP	Cowdung
Inorganic fertilizer only	15 – 20	3 – 4	3 - 4	-
Combination of organic and inorganic fertilizer	7.5	1.5	1.5	750

Table 2.5 Moisture, organic and mineral content of some organic wastes expressed in percent (%) dry matter (Gijzen and Khondker, 1997)

Nutrient Source	Moisture	Dry organic matter	C	N	P₂O₅	K₂O	CaO
Human faecal matter	65-80	88-97	40-55	5 - 7	3 - 5.5	1-2.5	4 – 5
Human urine	93 - 96	65 - 85	11 - 17	15 - 19	2.5 - 5	3 - 4.5	4.5 - 6
Urban refuse	10 - 60	25 - 35	12 - 17	0.4 - 0.8	0.2 - 0.5	0.8 - 1.5	4 - 7.5
Water hyacinth compost	85 - 95	-	-	1.9	1	2.9	4.6
Cow dung (fresh)	85	-	-	0.4	0.02	0.1	-
Cow dung (compost)	-	-	-	0.5	0.3	0.2	0.3
Pig manure (fresh)	80	-	-	0.5	0.5	0.45	-
Poultry (fresh)	-	-	-	1.6	1.5	0.85	-
Digester effluent charged with pig manure	-	6.5	-	3.4	-	-	-

2.3. NUTRITIVE VALUE

Appenroth *et al.* (2017) analysed the species of the duckweed genera *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia* for protein, fat, and starch contents as well as their amino acid and fatty acid distribution. Protein content spanned from 20 - 35 %, fat from 4 - 7 %, and starch from 4 -10 % per dry weight. The main reason for this high nutritive value is that the entire body of duckweeds is composed of non-structural, metabolically active tissue; and most photosynthesis is devoted to the production of protein and nucleic acids.

Hasan and Chakrabarti (2009) have opined that the nutritional content of duckweed is probably more dependent on the mineral concentrations of the growth medium than on the species or their geographical location. Water low in nutrients generally results in reduced nutritional content and slow growth in duckweeds. A summary of the nutritional composition of different species grown in different environmental conditions is presented in Table 2.6.

Duckweeds also store varying amounts of calcium as calcium oxalate crystals in the vacuole which in large doses may be toxic to duckweed consumers. Franceschi (1989) demonstrated that the calcium oxalate content of *Lemna minor* depends greatly on the calcium content of the water on which they are growing. Elevated calcium in water favours formation of calcium oxalate crystals, and their content can be lowered by growth on low-calcium medium. Therefore, it seems likely that placing duckweed on soft water for a reasonably short period could lower oxalate content significantly.

Table 2.6 Chemical analyses of various duckweed species grown under different environment conditions

Duckweed species	Country	Aquatic environment	Moisture (%)	Proximate composition ¹ (% dry matter)					Minerals (% dry matter)		Reference
				CP	EE	Ash	CF	NFE	Ca	P	
<i>L. aequinoctialis</i>	Nepal	Low-nutrient ² roadside pool	-	7.1	-	-	-	-	-	0.35	Koirala (2015)
<i>L. gibba</i>	USA	Low-nutrient ² lagoon	-	9.4	1.8	16.8	17.0	55.5	1.38	0.72	Culley <i>et al.</i> (1981)
<i>L. minor</i>	Bangladesh	Pond, nutrient status not specified	92.0	14.0	1.9	12.1	11.1	60.9	-	-	Zaher <i>et al.</i> (1995)
<i>L. minor</i>	Bangladesh	Ditch, nutrient status not specified	93.8	20.3-23.5	-	-	-	-	-	-	Majid <i>et al.</i> (1992)
<i>S. polyrhiza</i>	USA	Low-nutrient ² lagoon	-	13.1	2.5	13.3	16.1	55.0	1.21	0.56	Culley <i>et al.</i> (1981)
<i>S. polyrhiza</i>	Bangladesh	Ditch, nutrient status not specified	95.0	17.3-28.4	-	-	-	-	-	-	Majid <i>et al.</i> (1992)
<i>S. polyrhiza</i>	Nepal	Low-nutrient ² roadside pool	-	13.3	-	-	-	-	-	0.32	Koirala (2015)
<i>S. punctata</i>	USA	Low-nutrient ² lagoon	-	10.6	2.3	14.1	11.3	61.7	0.98	0.61	Culley <i>et al.</i> (1981)

Contd.....

Duckweed species	Country	Aquatic environment	Moisture (%)	Proximate composition ¹ (% dry matter)					Minerals (% dry matter)		Reference
				CP	EE	Ash	CF	NFE	Ca	P	
W. arrhiza	Bangladesh	Ditch, nutrient status not specified	91.2	14.9	-	-	-	-	-	-	Majid et al. (1992)
L. gibba	USA	High-nutrient ³ lagoon	-	36.3	6.3	15.5	10.1	31.8	1.81	2.60	Culley et al. (1981)
L. gibba	USA	Dairy cattle waste lagoon	-	38.5	3.0	16.4	9.4	32.7	1.00	1.60	Hillman and Culley (1978)
L. minima	USA	Source not specified	-	31.0	2.0	14.0	10.0	42.2	-	-	Shireman et al. (1977)
L. perpusilla	Thailand	Septage-fed earthen pond	94 -94.3	25.3- 29.3	3.8- 4.5	15.4 - 17.6	6.9- 7.6	-	-	-	Hassan and Edwards (1992)
S. oligorrhiza	USA	Dairy cattle waste lagoon	-	37.8	3.8	12.0	7.3	39.1	1.30	1.50	Hillman and Culley (1978)
S. oligorrhiza	USA	Treated waste water effluent	-	32.7	6.3	20.3	13.5	27.2	1.49	1.15	Culley and Epps (1973)

Contd.....

Duckweed species	Country	Aquatic environment	Moisture (%)	Proximate composition ¹ (% dry matter)					Minerals (% dry matter)		Reference
				CP	EE	Ash	CF	NFE	Ca	P	
<i>S. polyrhiza</i>	Thailand	Septage-fed earthen pond	91.0	23.8	3.8	18.3	11.7	42.4	-	-	Hassan and Edwards (1992)
<i>S. polyrhiza</i>	USA	High-nutrient ³ lagoon	-	39.7	5.3	12.8	9.3	32.9	1.28	2.10	Culley <i>et al.</i> (1981)
<i>S. polyrhiza</i>	USA	Dairy cattle waste lagoon	-	40.9	6.7	12.9	8.7	30.8	2.10	1.40	Hillman and Culley (1978)
<i>S. punctata</i>	USA	High-nutrient ³ lagoon	-	36.8	4.8	15.2	9.7	33.5	1.75	1.50	Culley <i>et al.</i> (1981)

¹ CP = Crude protein, EE = ether extract, CF = crude fibre, NFE = nitrogen free extract, Ca = Calcium, P = Phosphorus

² Low-nutrient water body contained less than 5 mg/l TKN

³ High-nutrient water body contained more than 30 mg/l TKN

2.4. TURION FORMATION

Each frond of *Spirodela polyrhiza* possesses two meristematic primordia in pockets at its proximal end. Depending upon environmental conditions, these primordia form either normal vegetative fronds, or the overwintering buds (turions, Latin *turio* = shoot), and like the daughter vegetative fronds the turions also contain two meristematic pockets from which new vegetative fronds can develop following germination (Kuhdorf *et al.*, 2014).

In experiments with *S. polyrhiza* under controlled laboratory conditions, the following turion - inducing factors have been revealed : low temperature (Perry 1968) ; abscisic acid (Steward, 1969; Smart *et al.*, 1995); shortage of nutrients such as phosphate (Appenroth *et al.*, 1989), nitrate (Sibasaki and Oda, 1979; Malek and Cossins, 1983) or sulphate (Malek and Cossins, 1983); exogenously applied sugars (Henssen, 1954; Newton *et al.*, 1978); and high light intensity (Jacobs, 1947; Appenroth *et al.*, 1996).

Perry (1968) studied different clones of *Spirodela* from northeastern United States, Puerto Rico and Argentina for turion formation under different light intensity, photoperiod, night temperature, day temperature, and concentration of nitrate in axenic cultures. The clones from Argentina and Puerto Rico did not form turions under any of the experimental conditions.

Perry and Byrne (1969) cultured four clones of *Spirodela* in 15 ml of modified Hoagland's solution (without copper and nitrogen sources) plus different concentrations (0.01 - 3 µg/ ml) of abscisic acid (ABA) in commercial growth chambers. The ABA in as low as 0.01 µg/ ml concentration induced turion formation in ABA sensitive clones within 10 days.

Smart and Trewavas (2006) investigated the production, growth, and development of ABA - induced turion of *Spirodela*. Addition of ABA to the culture of the duckweed resulted in growth inhibition at concentrations as low as 10^{-6} mol m⁻³, growth being completely arrested at 10^{-2} mol m⁻³. Around 10^{-4} mol m⁻³, ABA induced the production of turions. The turion tissues differed from normal fronds in three respects: (a) accumulation of numerous starch grains, (b) thick cell walls, and (c) large deposits

of tannins and anthocyanin pigment at the expense of the vacuolar expansion characteristic of the normal maturity pattern.

Appenroth and Nickel (2009) measured chemical composition and temperature of water in a pond in which the concentration of nitrate and sulphate (both in millimolar range) remained fairly constant throughout the year but phosphate concentration decreased from approximately 13 μM at the beginning of the growing season to 2 μM at the time of onset of turion formation. This concentration was used in experiments under controlled conditions together with the other outdoor data (photoperiod, and day and night temperatures) in subsequent experiments to investigate their role in the induction of turion formation. The findings indicated the decisive role of phosphate deficiency in inducing turion formation.

Olah *et al.* (2014) studied the growth of *Spirodela polyrhiza* under cadmium (a heavy metal that inhibits phosphate nutrition in plants) stress in axenic cultures. High cadmium concentration (0.080 mg/ l) decreased the relative growth rates of culture within 3 days, and the first turion was produced in 0.075 mg/ l cadmium concentration by the 5th day of treatment. The authors attributed induction of turion formation to cadmium stress that imbalanced phosphate uptake.

2.5. FISHMEAL REPLACER

The low fibre content and high nutritional value of duckweed makes it a quality feed or feed component for fish, ducks, chickens, pigs, young and lactating ruminants, and possibly also for humans [Jha and Pokharel (Bhattarai), 2015]. Duckweeds are fed to fish in fresh form as a sole feed or in combination with other feed ingredients, and intensive fish production with duckweed as a predominant feed has been reported by a number of authors (Hepher and Pruginin, 1979; Robinette *et al.*, 1980; Culley *et al.*, 1981; Landolt and Kandel, 1987; Skillicorn *et al.*, 1993).

Galkina Abdullaev and Zacharova (1965) reported that the grass carp showed more rapid growth when using duckweed than other feed materials. Porath and Koton (1977) noted that the weight of grass carp could be tripled (from 100 g to 300 g) within 50 days when feeding a mixture of *Lemna gibba* and *L. minor*.

Fasakin *et al.* (1999) reported that duckweed meal (*Spirodela polyrhiza*) can form up to 30 % of the total diet of Nile tilapia without significant effect on performance, compared to control without duckweed. However, inclusion levels above this level progressively decreased fish performance.

2.6. WASTEWATER TREATMENT

Solids are relatively easy to remove, what is most difficult to remove from the wastewaters are dissolved salts such as nitrates, phosphates and other nutrients, toxic metal ions, xenobiotic pesticides, and radionuclides. In this context, the basic concept of a duckweed wastewater treatment system is to farm local duckweed on the wastewater for the removal of minerals, organic contaminations and radionuclides (Davis *et al.*, 2002). Duckweed systems distinguish themselves from other wastewater treatment mechanisms in that they also produce a valuable, protein rich biomass as a by-product. When there are nutrients (particularly nitrogen and phosphorus) available in the wastewater, duckweed takes the nutrients from the wastewater to support its growth and to store the nutrients in its tissues.

In general, the duckweeds *Lemna* and *Spirodela* are more widely investigated for reclamation of municipal and metal contaminated industrial wastewaters than *Wolffia* and *Wolffiella*, and duckweeds in mixture have higher nutrient removal efficiency than those in monoculture (Zhao and Zhao, 2014).

Landolt and Kandeler (1987) reported that of all aquatic plants, duckweeds have the greatest capacity in assimilating the macro-elements: nitrogen, phosphorus, potassium, calcium, sodium and magnesium, however, this may not be supported by other literature sources (Reddy and DeBusk, 1985; DeBusk and Reddy, 1987). The data presented in Table 2.7 suggests that nutrient removal rates for duckweed are comparatively slower than for other aquatic plants and, therefore, longer retention times will be necessary to reduce nutrient concentrations to specific discharge limits.

Water hyacinth has been widely used for its extremely high nutrient uptake efficiency (Table 2.7). However, no economically attractive application of the harvested biomass has so far been identified. In addition, water hyacinth only grows efficiently in

tropical climates. A specific comparison of duckweed with water hyacinth for wastewater treatment and biomass use is presented in Table 2.8.

Table 2.7 Daily nitrogen and phosphorus uptake rates (g/m²/day) by different floating aquatic macrophytes (Reddy and DeBusk, 1985; DeBusk and Reddy, 1987)

Plant	Nitrogen		Phosphorus	
	Summer	Winter	Summer	Winter
Water hyacinth	1.30	0.25	0.24	0.05
Water lettuce	0.99	0.26	0.22	0.07
Pennywort	0.37	0.37	0.09	0.08
Duckweed (<i>Spirodela polyrhiza</i>)	0.15		0.03	

Table 2.8 Comparison between duckweed and water hyacinth for wastewater treatment and biomass use (Iqbal, 1999)

Criterion	Duckweed	Water hyacinth
Tolerance to low temperatures	Higher	Lower, more restricted to warm climates
Nutrient uptake capacity	<ul style="list-style-type: none"> • High, but smaller contact area with the wastewater surface • High tolerance to high nutrient concentrations 	Higher, due to greater contact area with the wastewater through root hairs
BOD removal efficiency	<ul style="list-style-type: none"> • Lower, because of smaller surface area for attached bacteria growth and lower oxygen supply. • Lower tolerance to high BOD concentrations (< 200 mg/l) 	<ul style="list-style-type: none"> • Higher because of larger surface area for attached bacteria growth and higher oxygen supply to the root zone. • Treatment of wastewater with very high BOD concentrations (> 1000 mg/l)
Removal capacity of organic xenobiotics and heavy metals	High	High
Mosquito control	Positive	Negative
Harvesting	<ul style="list-style-type: none"> • Easier • Can be done manually and mechanically 	<ul style="list-style-type: none"> • Complicated, because plants are bulky and interconnected over large distances • Mechanical harvesting equipment necessary

Continued.....

Nutrient profile (in % dry weight) when grown on wastewater	Protein (30 - 45 %)	• Protein (10 - 25 %)
	• Carbohydrate (35 %)	• Carbohydrate (35 - 72 %)
	• Fibre (7 - 14 %)	• Fibre (17 - 20 %)
	• Fat (3 - 7 %)	• Fat (1 - 3 %)
	• High vitamin and mineral content	
Use of biomass	• High quality feed supplement for fish and other animals	• Generally not consumed by fish and other animals
	• Land application	• Land application
	• Composting	• Composting
	• Methane and ethanol fermentation	• Biogas digestion
	• Medicinal plant	• Paper production
Water loss through evapotranspiration (ET)	Lower ET rates compared to open water (20 - 30 % reduction)	Equal or increased ET rates compared to open water

Response of *Lemna minor* grown in secondary effluent under conditions of artificial light and constant temperature was measured by Harvey and Fox (1973). The levels of total nitrogen and total phosphorus in the effluent decreased by 86.5 and 67 %, respectively, over a 10 - day period.

To arrive at detailed nutrient balances for duckweed - covered wastewater treatment systems, Korner and Vermaat (1998) carried out five laboratory - scale experiments in shallow (3.3 cm) 1 l batch systems and observed that duckweed - covered system removed 73 - 90 % of the initial Kjeldahl nitrogen and 63 - 99 % of the initial total phosphorus in 3 days.

Kuraishi and Sharma (2010) examined the efficiency of *Lemna minor* as an alternative cost effective natural biological tool in wastewater treatment. They inoculated *L. minor* into raw domestic sewage in a batch reactor over 28 day retention time under local outdoor natural conditions. Samples were taken below duckweed cover after every 7 day to assess the system's efficiency. The duckweed removed nutrients from the effluent satisfactorily and also restricted algal growth.

Nassar *et al.* (2015) used duckweed for agricultural drain wastewater purification and for producing an economic animal fodder rich in protein content. The achieved value of nutrient (phosphorus and nitrogen) removal from raw water in the effluent of duckweed pond operated at 10 days hydraulic detention times were 76.9 and 68.3 %, respectively. The contents of protein and phosphorus in the cultured duckweed were 28.1 and 0.83 %.

Mohedano and Filho (2012) evaluated the efficiency of two full-scale duckweed ponds for nutrient recovery from a piggery farm effluent (produced by 300 animals), and noted 98 % removal of total Kjeldahl nitrogen and 98.8 % removal of total phosphorus from the effluent.

Jain and Jha (1989) investigated the uptake of iron and copper by duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata* R. Br.) in solutions enriched with 1, 2, 4 and 8 ppm of these two metal ions. The uptake rate of both the metal ions was highest when the initial concentration in the test solution was 1 ppm.

Naumann and Appenroth (2007) used duckweed *Lemna minor* L. clone St to investigate the effect of 10 heavy metals under the standardised test conditions on frond number, fresh weight, dry weight, chlorophyll and carotenoid contents, and reported the following series of phytotoxicity: Ag > Cd > Hg > Ti > Cu > Ni > Zn > Co > Cr > As.

Khellaf and Zerdauli (2009) assessed the tolerance and effect of heavy metals on *Lemna minor* exposed to different concentrations of copper, nickel, cadmium and zinc in a quarter Coic and Lessaint solution at pH 6.1 under daily regime of 16 h light. Copper at 0.2 mg/l and nickel at 0.5 mg/l concentration promoted the growth of *Lemna* fronds but inhibited growth at higher concentrations.

Sekoma and Lens (2014) evaluated the use of algae and duckweed ponds as post - treatment for textile wastewater and reported small differences in metal (chromium, lead, cadmium and copper) removal efficiency between algal and duckweed ponds.

Verma and Suthar (2015) used three strengths of lead and cadmium (2, 5 and 10 mg/l) with varying pH (5, 7 and 9) and recorded the changes in metal concentration and metal uptake yield of the system. The lead and cadmium removal ranged between 60.1 (2 mg/l at pH 9) and 98.1 % (10 mg/l at pH 7), and 41.6 (10 mg/l at pH 9) and 84.8 % (2 mg/l at pH 7), respectively. The duckweed set up with pH 7 showed the optimum metal removal.

Ali and Ahmad (2016) recommended wastewater treatment at point pollution sources with duckweed plant species and suggested safe disposal of metal - contaminated duckweeds *via* incineration, carbonization, hydrolyzes, or anaerobic digestion for the restoration of the ecological equilibria in aquatic and terrestrial habitats that are already at stake by diverse anthropogenic activities.

2.7. DUCKWEED MANURE

Nutrients are essential for crop production. All plants require nutrients to grow and a significant portion of these nutrients are removed and exported when a crop is harvested. Sustainable crop production hence requires the nutrients that are removed to be replaced with inorganic and/or organic fertilizers. Inorganic fertilizers provide plant nutrients quickly so that the plants are able to get the nutrients as soon as possible. However, if nutrients are applied in excess of crop requirements and removal, they increase the risk of agronomic problems such as crop lodging (in the case of N), nutrient imbalances or toxicities and environmental problems such as nitrate leaching to groundwater, P runoff to surface water and release of greenhouse gases to the atmosphere. In contrast, the biggest advantage of an organic fertilizer is that the danger of over-fertilization is reduced as the nutrients are released slowly, are available for a longer period, and only a few applications are required. Further, the organic fertilizer adds more natural nutrients, feeds important soil microorganisms, and improves soil structure (Jha, 1990; Constance, 2010; Velten *et al.*, 2015).

With reference to some obsolete literature pertaining to the use of duckweeds as fertilizer and soil improver on field and gardens in Angola, China and Mexico, Lot *et al.* (1979) inferred that the application of duckweed eventually contributed to a superior soil texture, including an improved water and cation exchange, and resulted in an annual harvest of 4 crops of vegetables or corn. The authors opined to use duckweed as an organic fertilizer in agriculture by direct land application or *via* composting.

Mahofa *et al.* (2014) investigated the effects of green and composted (including sawdust, chicken droppings and soil) duckweed manure on height and yield of Floradade tomatoes. Both types of duckweed manure significantly increased the growth and yield of tomatoes, though green manure (C : N ratio 20 : 1) promoted higher growth and yield than the compost (C : N ratio 400 : 1).

Lin *et al.* (2017) conducted a field experiment for three years with five treatments (without N-fertilizer; conventional practice, urea alone at 300 kg N/ha; urea combined with duckweed at 300 kg N/ha; urea alone at 225 kg N/ ha ; urea combined with

duckweed at 225 kg N/ ha). The results showed that urea combined with duckweed-cover reduced NH_3 loss by 36 - 52 % over conventional practice. Furthermore, urea combined with duckweed increased N accumulation in the aboveground plants by 14 - 25 % over conventional practice for the 3 years. As a result, urea combined with duckweed achieved higher rice yield by 9 - 10 % and higher economic benefit by 10- 11 % over conventional practice for 3 years ; however , using conventional rate of 300 kg N/ ha did not increase rice yield over using the reduced N rate of 225 kg N/ ha with or without duckweed.

In light of the current scientific findings and opinion, duckweeds are bound to be a new commercial crop worldwide in the coming days. There are hundreds of derelict ponds polluted to eutrophication levels at Biratnagar alone that could be resurrected for fish farming through duckweed aquaculture. Further, duckweed aquaculture could also cleanse water bodies contaminated with the highly toxic heavy metal mercury used in gold-plating works, dental amalgam, cosmetics, pharmaceuticals, etc. and disposed of haphazardly. In addition, duckweeds constitute a proteinous feed for fish and livestock farming, and also could be used as noble organic fertilizer in agriculture as well as a cheap source for ethanol production. However, previous works in Nepal are limited to the distribution of duckweeds in the country only (Hara *et al.* 1978, Upadhyay *et al.*, 2011; Koirala, 2015).

CHAPTER 3
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. STUDY SITE

Biratnagar - the industrial capital of Nepal is a metropolitan city and the interim capital of the Province Number 1 of Nepal (Fig. 3.1). It is currently the second most densely populated city (after Kathmandu) of Nepal, with a population of 240000 (GoN, 2019). The city has a total area of 77.5 sq km, a geographical location of 26⁰28' N latitude and 87⁰16' E longitude, 72 m msl altitude, and is located in the Morang District of the south-eastern Terai region of Nepal. It lies 399 km east of Nepal's capital, Kathmandu, and 6 km north of the Jogbani boarder of the Indian state of Bihar.

Soil of Biratnagar is made up of materials transported and deposited in relatively recent times by the Sapta Koshi River and its tributaries (Singhia and Keshaliya). Being immature, the soil has week profile. The overall textural composition of the soil is: sand 39 %, silt 46 %, and clay 15 %; and maximum value of soil pH (5.7), phosphorus (0.005 %), and potassium (0.073 %) occur in January (winter), and those of organic carbon (3.35 %), nitrogen (0.22 %), and C/N ratio (15.22 : 1) in May (summer season) in top 10 cm of soil layer (Jha, 2010).

The study site experiences three distinct seasons, *viz.* monsoon (June to October), winter (November to February), and summer (March to June). The south - east monsoon arising from the Bay of Bengal generally sets in the first week of July and continues till October, and Biratnagar receives most of the rains (1299.7 mm or 68.7 %) during this period. The average minimum and maximum aerial temperatures are 24.3 and 32.4 °C, respectively, whereas mean temperature is 28.2 °C. Average relative humidity is 87.8 %. Biratnagar receives the least rainfall (37.4 mm or 2 %) during the winter season, and mean temperature and relative humidity are 18.70 °C and 87 %, respectively. During the summer, Biratnagar receives 555 mm rainfall and rains are generally accompanied by thunderstorms. The city often experiences a blast of hot air ('Loo') during this season. The general trend of wind velocity (m/s) at Biratnagar is 0.12 m/s in January and 2.08 m/s in May (Siwakoti and Verma, 1999).

Forests in the adjoining areas of Biratnagar are dominated by *Shorea robusta* Gaertn. With *Adina cordifolia* (Roxb.) Brandis, *Cassia fistula* L., *Dillenia pentagyna* Roxb., *Emblica officinalis* Gaertn., *Lagerstroemia parviflora* Roxb., *Mallotus philippensis* (Lam.) Mull. Arg., *Schleichera oleosa* (Lour.) Oken, *Semecarpus anacardium* L. f., *Terminalia alata* Heyne ex Roth, *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., and *Toona ciliata* M. Roem. as the main associates (Jha and Jha 2000). Paddy and wheat are staple crops whereas sugarcane and jute are cultivated as cash crops.

Among the wetland flora of Biratnagar, notable free-floating species include *Azolla imbricata* (Roxb.) Nakai, *Eichornia crassipes* (Mart.) Solms, *Lemna aequinoctialis* Welwitsch, *Pistia stratiotes* L. and *Spirodela polyrhiza* (L.) Schleiden, whereas common floating-leaved species are *Ludwigia adscendens* (L.) H. Hara, *Nelumbo nucifera* Gaerten., *Nymphaea pubescens* Willd, *Nymphoides hydrophyllum* (Lour.) Kuntze and *Trapa quadrispinosa* Roxb. The common submerged macrophytes are *Ceratophyllum demersum* L., *Hydrilla verticillata* (L. f.) Royle, *Ottelia alismoides* (L.) Pers. and *Potamogeton Crispus* L. ; whereas *Alternanthera philoxeroides* (Mart.) Griseb., *Arundinella bengalensis* (Spreng.) Druce, *Cyperus pilosus* Vahl, *Ipomoea carnea* Jacq. subsp., *fistulosa* (Mart. ex Choisy) D. F. Austin, *Leersia hexandra* Sw., *Monochoria hastata* (L.) Solms, *Phragmites karka* (Retz.) Trin. ex Steud. and *Typha domingensis* Persoon are the ubiquitous emergent macrophytes.

Table 3.1 Climate data for Biratnagar Airport (1989 - 2018) (Department of Hydrology & Meteorology, GoN, 2019)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Maximum temperature (°C)	22.7	26.1	30.9	33.9	33.3	32.9	32.1	32.5	32.1	31.6	29.3	25.4	30.2
Minimum temperature (°C)	9.0	11.1	15.6	20.4	23.3	25.2	25.6	25.8	24.7	21.1	15.3	10.5	19.0
Mean temperature (°C)	15.9	18.6	23.3	27.2	28.3	29.1	28.9	29.2	28.4	26.4	22.3	18.0	24.6
Rainfall (mm)	11.7	13.2	13.2	53.1	186.0	302.4	530.8	378.3	298.8	91.8	5.9	6.6	1891.8
Relative humidity (%)	95.8	87.9	80.2	68.6	75.5	86.1	88.6	92.0	87.5	83.0	79.5	84.3	84.1

3.2. *IN – SITU GROWTH*

In the present study, seasonal variation in biomass and nutritive value of *S. polyrhiza* occurring in a eutrophic pool was investigated in 2015, whereas monthly variation in morphology in different populations of the plant was observed during December 2016 and May 2017 (Fig. 3.1).

3.2.1. *Seasonal Variations in Biomass and Nutrients*

Seasonal variations in biomass and nutritive value (protein, phosphorus and potassium) of *S. polyrhiza* along with the physico-chemical properties of the habitat water (temperature, pH, total dissolved solids, nitrogen, phosphorus and potassium) were studied in a 40 m long and 3.5 m wide pool (Plate 2) with water depth in the centre 0.45 m in summer and 1.12 m in the rainy season. The pool, located 200 m west to Bigyan Bhawan (PG Campus), received nutrients all year round through domestic wastewater and agricultural runoff, and the shore area (0 - 0.2 m water depth) vegetation of the pool was composed of *Alternanthera philoxeroides* (Mart.), *Arundinella bengalensis* (Spreng.) Druce, *Colocasia esculenta* (L.) Schott, *Cyperus pilosus* Vahl, *Eichhornia crassipes* (Mart.) Solms, *Ludwigia adscendens* (L.) H. Hara, *Mikania micrantha* Kunth, *Persicaria glabra* (Willd.) M. Gomez, *Rumex dentatus* L. and *Sacciolepis indica* (L.) Chase; whereas *Lemna aequinoctialis* Welwitsch, *Nitella mucronatus* (Thuill) Kuentz, *Potamogeton crispus* L. and *Spirodela polyrhiza* (L.) Schleiden occupied deep water (> 0.2 m depth) zone. The base - layer soil (mud) had 49 % silt, 26 % sand, 25 % clay, 0.25 % nitrogen, 0.001 % phosphorus, 0.076 % potassium, 3.96 % organic carbon, and 15.84 : 1 C : N ratio (Koirala, 2015).

Intermixed *S. polyrhiza* and *Lemna aequinoctialis* were sampled using ten 10 cm x 10 cm quadrat randomly in the first week of January (Winter), May (Summer) and September (Rainy Season), 2015, along with the habitat water for physico-chemical analyses. The collected duckweeds were separated species-wise, and *S. polyrhiza* fronds were squeezed gently between blotting papers before weighing for fresh biomass. These fronds were dried in oven at 80°C to a constant weight for nutrient determination.

3.2.2. Morphological Variations

Three roadside pools (Plate 3) receiving domestic sewage all year round were selected for the study of monthly variations in number (per unit area), morphology (frond size and root length), and biomass of *S. polyrhiza* between December 2016 and May 2017 (a downpour in the last week of May 2017 washed away most of the fronds from the selected pools, thus hindering further investigation).

Among the selected pools, the first one was located in the vicinity of Biratnagar bus - park (water body 1 or WB 1); water body 2 (WB 2) was situated adjacent to the Koshi - project complex along Biratnagar - Jogbani (Rani) road; and water body 3 (WB 3) in the vicinity of the National Homeopathy College along Balbhadra highway. Average water depth in the centre of all the selected pools was about 1 m in December and 0.3 m in May.

Water and duckweed mixture were sampled randomly in the first week of each month from all the selected pools using 10 cm x 10 cm quadrat (fitted with a sieve and handle) in triplicate. The duckweed fronds were separated species-wise for determination of frond number and biomass (per unit area) of *S. polyrhiza*. Besides, 50 *S. polyrhiza* fronds selected randomly were measured for size (graphically) and root length.

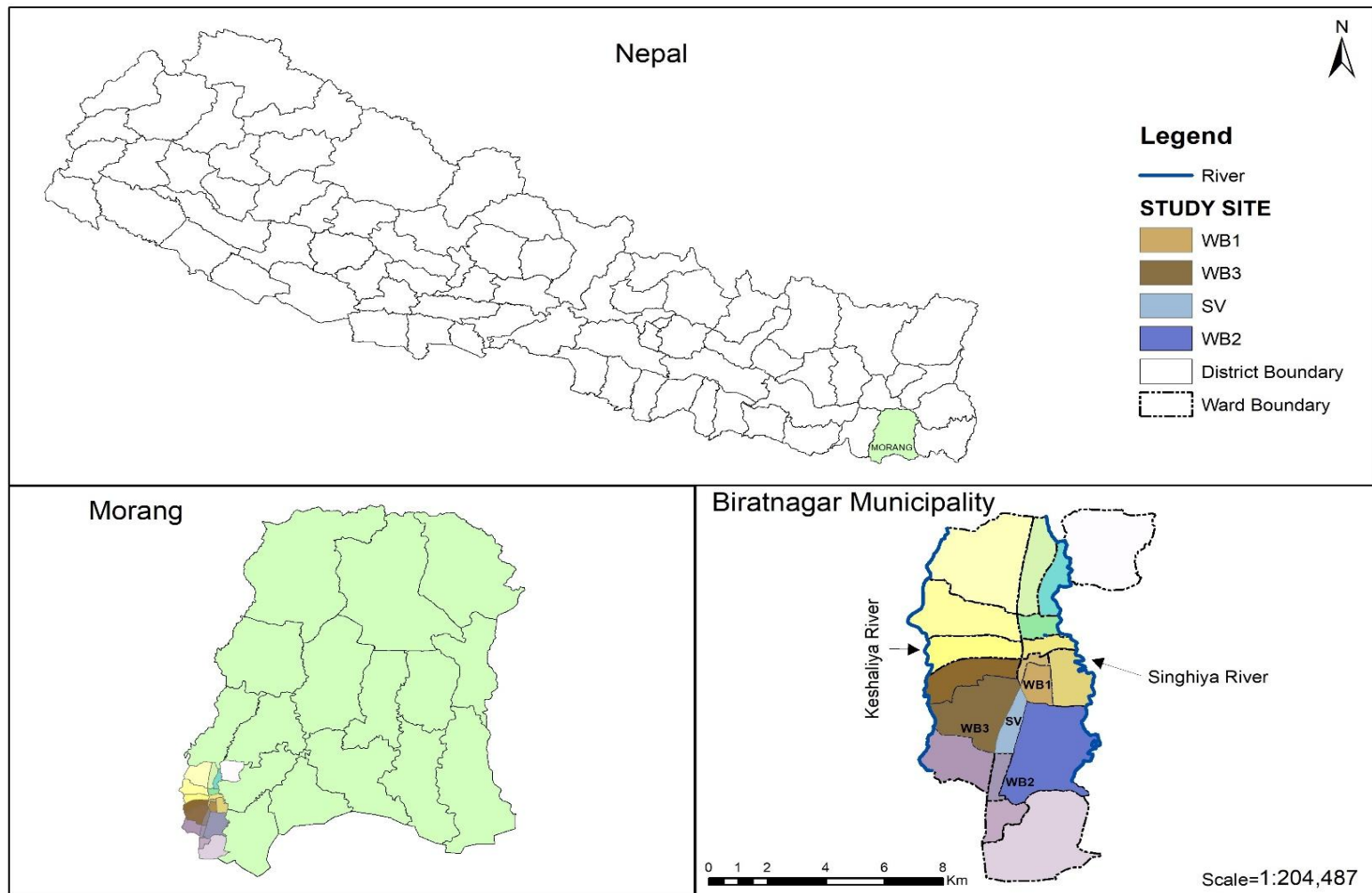


Figure 3.1 Map of the study site; SV = habitat to study seasonal variations in biomass and nutrients of *S. polyrhiza*; WB1 - WB3 = pools selected to study morphological variations in fronds of *S. polyrhiza*



Plate 2 Researcher sampling duckweed mixture with quadrat in the habitat selected for investigating seasonal variations in biomass and nutrients in *Spirodela polyrhiza*



WB 1



WB 2



WB 3

Plate 3 Selected pools to investigate morphological variations: WB 1 = Water body 1 (adjacent to Biratnagar Bus Park); WB 2 = Water body 2 (adjacent to Western gate of Koshi-project complex along Biratnagar-Rani road; WB 3 = Water body 3 (adjacent to the National Homeopathy College)

3.3. GROWTH ON DIFFERENT WATER pH AND NUTRIENT MEDIA

Garden soil (2 kg/ pot) was placed in 27 earthen pots (with sealed drainage) of 20 cm diameter and 15 cm depth, and all the pots were flooded with unchlorinated borewell water. Such pots were arranged in nine different groups (each group in triplicate) of water pH (2, 3, 4, 5, 6, 7, 8, 9, 10) either through adding hydrochloric acid or lime, and left overnight. *Spirodela polyrhiza* fronds were collected from an eutrophic pool located near PG Campus, washed in running tap water, and were spread in each pot at the rate of 1 g fresh fronds per pot. The pots were kept in open and watering was done regularly to compensate for evaporative losses. The fronds were harvested after 30 days of incubation (December 29, 2015 to January 27, 2016), and their fresh weight per pot was taken immediately after harvest. Similarly, mixture of 1 g fresh *S. polyrhiza* fronds and 1 g fresh *Lemna aequinoctialis* fronds were also incubated on different water pH between January 29 and February 27, 2016. Species-wise these fronds were separated from each other after the completion of incubation period (30 days), and increase/ decrease in their fresh weights (prior to incubation) were measured.

Different concentrations of inorganic fertilizer (combination of urea, diammonium phosphate and muriate of potash in 5 : 1 : 1 ratio), and sugars (glucose, sucrose, xylose) were made in borewell water. Three l solution of each strength in triplicate was kept in plastic bowls along with a control (borewell water only) under laboratory conditions, and 1 g fresh *S. polyrhiza* fronds of uniform size were incubated in all the bowls. Borewell water was added into the bowls at regular intervals to compensate for the evaporative losses. One fortnight after the commencement of each experiment, the inoculated fronds were harvested for measuring increase/ decrease in their fresh weight. Growth experiments on different media were conducted as per the following schedule : February 28 to March 14, 2017 (inorganic fertilizer) ; April 8 - 22, 2017 (sucrose) ; April 23 to May 7, 2017 (glucose) ; May 10 - 24, 2017 (xylose).

3.4. TURION FORMATION

Normal fronds of *S. polyrhiza* were collected from an eutrophic pool, washed thoroughly, and incubated on borewell water (3 l) in plastic bowls in triplicate at the rate of 50 fronds per bowl on December 2, 2018. Similarly, *S. polyrhiza* fronds were also incubated in triplicate on habitat water simultaneously. The bowls with incubated

S. polyrhiza were kept in open on the roof top of PG Campus, and fronds were inspected daily until turions became visible. Water level in the bowls was maintained throughout the observation period with addition of either fresh borewell or habitat water. Triplicate samples of the borewell water as well as those from the eutrophic pool harbouring *S. polyrhiza* fronds were examined for differences in pH, nitrogen, phosphorus and potassium levels after turions were visible.

3.5. FISHMEAL REPLACER

The tilapia (*Oreochromis niloticus* L.) fingerlings (length 9.97 cm, breadth 2.96 cm and weight 13.43 g) were obtained from the Fisheries Section of the Nepal Agricultural Research Council (Tarhara branch, Nepal) and brought to the Bigyan Bhawan (P G Campus, Biratnagar) in polythene bags partially filled with water and oxygen. They were released into a 5m x 2m concrete tank for acclimatization for two days. Nine transparent glass aquaria (size 45 cm x 30 cm x 30 cm) filled up to two - third volume with borewell water and having aeration facilities were employed for rearing tilapia fingerlings (3 individuals per aquarium) in three sets. In the first set of three aquaria, the fish received only the conventional (CF feed ; in the second set of aquaria the fingerlings received a mixture (1 : 1 ratio) of CF and *S. polyrhiza* powder (GD); whereas only GD was provided to the fish in the third set of aquaria. Specified feed was provided daily at the rate of 20 % of the body weight of the fish following Kabir *et al.* (2009). Water in each aquarium was replaced with fresh borewell water at 2 - day intervals.

Among the feeds, CF was prepared by mixing wheat flour, soybean (*Glycine max* (L.) Merr.) flour and powdered local fishes in 2 : 2 : 1 ratio, whereas for GD, *S. polyrhiza* biomass, collected from a municipal sewage - fed pool adjacent to the PG Campus, was washed, dried in oven at 80 °C to a constant weight, and grinded to powder in an electric grinder.

The experiment was conducted between November 3 - 24, 2017 during which the minimum and maximum atmospheric temperatures were 15.5 and 30 °C, respectively. The nutritive value (proteins, fats, ash, calcium and phosphorus) of the three feeds as

well as accumulation of nutrients in fish tissues reared on different feeds were also measured.

3.6. WASTEWATER TREATMENT

Duckweed's nutrient removal efficiency from polluted water and response of duckweed to heavy metal (mercury) stress were investigated in the present study.

3.6.1. Water Cleansing Efficiency

Water samples were collected from a ditch located in the main vegetable market (Gudari) of Biratnagar on August 31, 2017. The ditch was used by vendors for dumping spoiled vegetables. These water samples were analysed for pH and nutrients (nitrogen, phosphorus and potassium) before incubating *S. polyrhiza* fronds on them. Thereafter, 3 l ditch water was placed in plastic bowls in triplicate, and 1 g fresh *S. polyrhiza* fronds were incubated on them. Water level in the bowls was maintained through addition of borewell water throughout the incubation period, and the cultured fronds were harvested one month after commencement of experiment for measuring changes in fresh weight. The residual amount of nutrients in wastewater was measured in triplicate after the fronds were harvested, and the mean values of nutrients present in wastewater before and after incubating *S. polyrhiza* were compared.

3.6.2. Response to Mercury Stress

Three l solution of each strength (2, 4, 6, 8, 10 mg/ l) of mercuric chloride dissolved in borewell water were kept in triplicate in plastic bowls along with control set (borewell water only) under laboratory conditions and 5 g fresh *S. polyrhiza* fronds were incubated in each bowl on July 7, 2018. The fronds were harvested one week after incubation for the measurement of increase/ decrease in fresh weight as well as photosynthetic pigments (chlorophylls and carotenoids).

3.7. APPLICATION OF DUCKWEED MANURE

The mixture of *Spirodela polyrhiza* and *Lemna aequinoctialis* was collected from a sewage-fed pool through nylon net, squeezed and kept in five 15 l air-tight plastic containers for two months at room conditions on October 7, 2015, for the preparation of the semi-liquid duckweed manure.

Forty earthen pots of 25 cm diameter and 20 cm depth, filled up to three-fourth volume with the kitchen garden soil (texture : sand 39 %, silt 46 %, clay 15 %; pH 6.6 ; organic matter 6.17 %; N 0.32 %; P 5.01 ppm; K 60.42 ppm) were employed for each test species (chilli and brinjal crops). For manure treatment, each test species was segregated into four sets of 10 pots each as follows : (i) Control (C), to receive no manure, (ii) 7 – D, to receive 500 ml manure at 7 – day intervals (iii) 14 – D, to receive 500 ml manure at 14 – day intervals, (iv) 21 – D, to receive 500 ml manure at 21 – day intervals. The pots for 7 – D, 14 – D and 21 – D treatments received first application of 500 ml duckweed manure on December 7, 2015, and thereafter manuring was done on specified dates. All the pots received 2 l water at every 4 – day intervals from the date of seedling planting, and weeds and pests were controlled till the harvest of the test species.

Measurements of height, number of leaves, leaf area, and branch number per individual under different frequencies of manuring and control commenced 46 days after plantation of the test species. Fruit picking was carried out on April 11, 2016 (when over 80 % fruits had reached maturity) and again on April 18, 2016. The plants were harvested between April 18 - 20, 2016 for the final measurements of height, branch number, leaf number, leaf area, and dry weights of leaf, stem, and root per plant.

Composite soil samples from control and manure treated pots were analysed for pH, organic matter, N, P and K to ascertain the ameliorative effects of duckweed manuring on these parameters.

3.8. PHYSICO - CHEMICAL ANALYSES

3.8.1. Water

The water samples were collected in triplicate from the pool harbouring the *S. polyrhiza* colony by lowering 500 ml glass bottles to 20 cm below the duckweed cover with stopper in place, followed by opening of bottles to be filled by water samples. After filling with water samples, the bottles were closed with stopper, and raised to the surface, and brought to the laboratory. Water samples were analysed immediately for

the pH, and stored at 4 °C in dark after adding sulphuric acid to reduce the sample pH to less than 2 (APHA, 1990).

Temperature and pH: Temperature was noted by dipping a mercury thermometer 20 cm below the water surface in habitat for 2 min, whereas pH was determined by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The pH meter was stabilized for 10 min, and calibrated with standard buffer solutions (pH 4, pH 7 and pH 9 buffer tablets). The electrode was washed with distilled water, wiped out with tissue paper gently, and it was immersed into the beaker containing the water sample for pH determination.

Total dissolved solids (TDS): TDS of water samples was measured following the method of US Geological Survey (1974). A well - mixed water sample was filtered through a standard glass fiber filter (without organic binder). Two hundred ml of the filtered sample was pipetted into a weighed porcelain evaporating basin (a). It was evaporated at 110 °C and dried further for 1 h after evaporation at 180 °C in the oven. Thereafter the basin was cooled in a dessicator and weighed (b). Calculation was done using the formula:

$$\text{TDS (mg/l)} = (\text{b} - \text{a}) \times 1000 / \text{V}$$

where, a = weight (g) of the empty basin;

b = weight (g) of basin plus residues;

V = total volume (ml) of filtered sample taken for evaporation.

Total nitrogen: Total nitrogen of the water samples was measured by macro Kjeldahl method (APHA, 1990). For the preparation of digestion mixture, 10 g copper sulphate was grinded and mixed with 200 g sodium sulphate. Sodium hydroxide solution (40 %) was prepared by dissolving 400 g sodium hydroxide in 1 l distilled water and this solution was cooled further. Mixed indicator was prepared by dissolving 0.5 g bromocresol green and 0.1 g methyl red in 100 ml of 95 % ethanol. Four percent boric acid was prepared by dissolving 40 g boric powder in 1 l warmed distilled water. Standard hydrochloric acid (0.05 N) was prepared from 1 N hydrochloric acid by dilution method. For the preparation of standard sodium carbonate solution (0.05 N),

2.5 g anhydrous sodium carbonate was dissolved in distilled water to make 1 l volume.

Two hundred ml of sample water was pipetted into 500 ml breaker to which 2 ml concentrated sulphuric acid was added. Thereafter, water sample was digested over a hot plate until the volume was reduced to 10 ml. It was cooled and transferred to Kjeldahl digestion flask to which 1 g digestion mixture and 10 ml concentrated sulphuric acid was added. Thereafter, the digestion flask was placed over a heater till copious white fumes commenced. Heating was continued further for 30 min. Thereafter, the digestion flask was cooled and 200 ml distilled water was added to the content. Next, the content of digestion flask was transferred into a distillation flask to which 40 ml of 40 % sodium hydroxide solution was added. This mixture was distilled and liberated ammonia was allowed to be absorbed in 25 ml of 4 % boric acid until the distillate volume reached about 200 ml. A few drops of bromocresol green indicator was added to distillate and it was titrated against standard 0.05 N hydrochloric acid until the end point. Similarly, a blank was ran with distilled water in triplicate. The nitrogen concentration in water sample was calculated as:

1 ml of 1 N HCl = 14 mg of nitrogen

Hence, nitrogen (mg/l) = $14 \times v \times N \times 1000 / V = y$

where,

v = Deducted volume

(i.e., volume consumed by the sample minus volume consumed by blank) (ml),

N = strength of standard sodium hydroxide solution,

V = volume of sample taken (ml).

Total phosphorus: Total phosphorus of water samples was measured using vanadomolybdo-phosphoric acid colorimetric method (APHA 1990). For preparation of vanadate-molybdate reagent 25 g ammonium molybdate was dissolved in 300 ml warm distilled water and left for cooling. It was treated as *solution A*. Next, ammonium metavanadate (1.25 g) was dissolved in 300 ml boiled distilled water and left for cooling. After cooling, 330 ml concentrated hydrochloric acid was added to ammonium metavanadate solution and left for cooling. It was treated as *solution B*. The

cooled *solution B* was mixed with *solution A* and total volume was made 1 l using distilled water.

For the preparation of standard phosphorus solution, 0.2195 g dried KH_2PO_4 was dissolved in distilled water and acidified with 25 ml of 7N sulphuric acid. The volume of this standard phosphorus solution was made 1 l using distilled water to get 50 mg/ l phosphorus solution.

Twenty five ml water sample was placed in a volumetric flask to which 2 - 3 drops of phenolphthalein indicator was added; if red colour appeared 1:1 HCl – H_2SO_4 was added till the colour disappeared. To this treated water sample, 10 ml of vanadate-molybdate reagent was added, mixed well and diluted to 50 ml. The concentration of phosphorus in water sample was read at 840 nm wavelength after 10 min using spectrophotometer. The spectrophotometer was calibrated by taking 0, 1, 2, 3, 4 and 5 ml of 50 mg/ l phosphorus in separate 50 ml volumetric flask and in each volumetric flask 10 ml of the vanadate-molybdate reagent was added and final volume was made 50 ml by adding distilled water.

Phosphorus in water sample was calculated as:

$$P \text{ mg/l} = \frac{R \times 50}{25}$$

where, R was the reading of spectrophotometer.

Potassium: The potassium content of water samples was determined by Atomic Absorption Spectrophotometer (mode ICP 3000, USA ; APHA, 1990) . One hundred ml water sample was taken into a beaker to which 2 ml concentrated nitric acid was added. Thereafter, the beaker was placed on a hot plate at 120 °C for digestion till the volume in beaker was reduced to 5 ml. Then the beaker was cooled and 50 ml distilled water was added before transferring the content of the beaker into a volumetric flask for measuring potassium content in the Atomic Absorption Spectrophotometer.

3.8.2. *Spirodela*, Fishmeals and Fish Tissues

Fresh *S. polyrhiza* fronds were used for the extraction of photosynthetic pigments (chlorophylls and carotenoids), whereas for nutrient analyses, the fronds were dried to a constant weight at 80 °C in oven, and grinded. For analyzing nutrient accumulation in Nile tilapia reared on different feeds, scales, fins, head, bones and other inedible parts of the carcass were removed and the remaining flesh was dried in oven at 80 °C to a constant weight, and grinded. In general, the sample amount used for analyzing various nutrients was similar for grinded *S. polyrhiza*, different fishmeals, and fish tissues.

Chlorophylls and Carotenoid: Lichtenthaler' (1987) methods were used for estimation of both the pigment. Fresh *S. polyrhiza* fronds (1 g) was grinded in a mortar and some quantity (5 ml) of 90 % acetone (made basic by adding a few ml of magnesium carbonate) was added. The material was thoroughly crushed and then centrifuged at 250 rpm for 10-15 min. All the operations were carried out in shade without delay. The extract was decanted to a volumetric flask and the volume was made up to 50 ml using 90 % acetone. The chlorophyll concentration was then determined through recording absorbance in spectrophotometer at 663 and 645 nm .

$$\text{Chl a (mg/ g)} = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645})] \times V / 1000 \times W$$

$$\text{Chl b (mg/ g)} = [22.9 (\text{OD}_{645}) - 4.68 (\text{OD}_{663})] \times V / 1000 \times W$$

$$\text{Total Chl (mg/ g)} = \text{OD}_{645} \times 1000 / 34.5 \times V / 1000 \times W$$

where,

OD = optical density, V = volume of the acetone - chlorophyll extract, W = fresh weight of *S. polyrhiza* fronds.

For the estimation of carotenoid pigments, OD was recorded at 510 and 480 nm, and pigment concentration was calculated as;

$$\text{Carotenoid (mg/ g)} = [7.6 (\text{OD}_{480}) - 1.49 (\text{OD}_{510})] \times V / 1000 \times W$$

Nitrogen: Two hundred mg test material was transferred into a micro - Kjeldahl digestion flask to which 2 ml distilled water was added. The flask was shaken for 5 min and allowed to stand for 20 min. Thereafter, 8 ml sulphuric acid was added to the flask which was shaken further for thorough mixing of acid with the test sample. The flask was heated gently until fumes appeared, and removed for cooling. After cooling,

a pinch of catalyst (mixture of potassium sulphate and powdered metallic selenium in 50 : 1 ratio) was added into the flask, and the sample was digested first over a low flame and after the frothing ceased, under strong flame until colourless solution was formed. Digestion was continued for further half an hour. Simultaneously, a blank was run in the same manner without the test sample (10 ml distilled water) in another flask.

After cooling, volume of the digest was raised to 20 ml with addition of distilled water and it was treated as stock solution. One ml of stock solution was transferred to a test tube to which 14 ml distilled water and 1 ml of 6 N sodium hydroxide solution were added. The test tube was cooled in ice bath for 30 min. Then, 1.5 ml of Nessler's reagent was added to the tube and absorbance / optical density (OD) of the digest was read at 490 nm in a Spectronic-20 (Milton and Roy) spectrophotometer.

A standard nitrogen solution was prepared by dissolving 66 mg of ammonium sulphate in 100 ml of distilled water. From that solution, 0.2, 0.4, 0.6, 0.8 and 1 ml (representing 28, 56, 84, 112 and 140 $\mu\text{g N/ml}$, respectively) was taken into separate tubes and their volumes were adjusted to 15 ml by addition of distilled water. One ml 6N sodium hydroxide and 1.5 ml Nessler's reagent was added to each tube, and OD was recorded in spectrophotometer at 490 nm. A calibration curve using OD was drawn against concentration of nitrogen (μg) in ammonium sulphate solutions, and OD of the sample digest was tallied with the calibration curve to determine nitrogen or protein (protein = $\text{N} \times 6.25$) content of the samples.

Total ash: Five g powdered test sample was kept into a silica basin (previously ignited to a constant weight) and charred over a Bunsen burner. Thereafter, the basin with charred sample was transferred to a muffle furnace. Temperature of the muffle furnace was maintained at about 300 °C, till the sample ceased to glow and then the temperature was raised to 450 – 500 °C. Ignition was continued till a white ash was obtained. Thereafter, the basin was cooled in a desiccator, and ash was weighed.

Phosphorus: Phosphorus was estimated by the method suggested by Furman (1962). Five hundred mg powdered sample was transferred into a micro - Kjeldahl digestion flask to which 5 ml of 0.1 N magnesium nitrate, 4 ml perchloric acid, sufficient nitric

acid (about 35 ml) and 5 ml sulphuric acid were added successively. The flask was shaken for 5 min to mix the contents and heated gently at low heat until dense brown fumes appeared. Thereafter, the flask was removed from the heater for cooling. The cooled flask was replaced on heater and digestion was continued at low heat till white fumes appeared. Digestion was continued for another 10 min after the appearance of white fumes, and finally the flask was subjected to high heat until its content became colourless. The digest thus obtained was cooled and its final volume was raised to 100 ml by addition of distilled water.

Fifteen ml digest of the sample was pipetted into a 50 ml volumetric flask to which 2 ml of 10 N sulphuric acid was added. The solution was diluted further to 45 ml by addition of distilled water followed by addition of 1 ml of ammonium molybdate solution. The flask was shaken for 5 min, and 1 ml stannous chloride solution was added into the flask. The flask was shaken again for 5 min and final volume of the contents of flask was made 50 ml after adding appropriate amount of distilled water. After 20 min, the absorbance/ optical density of the digest was read at 660 nm in Spectronic-20 spectrophotometer.

For the preparation of standard phosphate solution, 4.388 g dried anhydrous potassium hydrogen phosphate was dissolved in 1 l distilled water. Ten ml of that solution was pipetted into a volumetric flask and diluted again to 1 l by addition of distilled water. It was standard phosphate solution containing 10 mg phosphorus/ l. To prepare a standard curve, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml aliquots of the above solution were diluted separately to 500 ml with distilled water to represent 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mg phosphorus/ l, respectively. Fifteen ml of these working standard phosphate solutions were pipetted separately into 50 ml volumetric flasks, and they were processed similarly to the test solution for determination of their optical density in spectrophotometer.

Potassium: Potassium was estimated by flame photometer method (Piper, 1944). For the preparation of standard stock solution of potassium, 1.907 g potassium chloride was dissolved in 1 l distilled water representing 1000 ppm potassium. Working standard potassium solution was prepared by diluting 10 ml of 1000 ppm K into 100 ml in a volumetric flask. From this working standard solution, 0, 1, 2, 4, 6, 8 and 10

ml solutions were taken in separate volumetric flasks to which 10 ml of 1 N nitric acid and distilled water were added to make final volume 100 ml that represented 0, 1, 2, 4, 6, 8 and 10 ppm potassium in the acid system. These were subjected to flame photometer reading, separately. In essence, the flame photometer readings 0, 10, 15, 35, 55, 75 and 95 represented 0, 1, 2, 4, 6, 8 and 10 ppm potassium concentration, respectively.

Five hundred mg powdered test sample was transferred into a micro-Kjeldahl digestion flask to which 4 ml perchloric acid, 35 ml nitric acid and 5 ml sulphuric acid were added successively. The procedure of sample digestion, in general, was similar to that for phosphorus. For the flame photometer reading five ml of digest of the sample was pipetted into a 50 ml volumetric flask and final volume was made 50 ml by adding distilled water. The sample value of potassium was calculated with the help of standard curve as:

$$\% \text{ K in test sample} = \text{ppm K from curve} \times \text{dilution factor} / 10^4$$

Calcium: Calcium content of the test samples were estimated following the method of Peach and Tracey (1956). Five g powdered test sample was transferred into a 300 ml Kjeldahl flask to which 4 ml perchloric acid, sufficient nitric acid (about 35 ml) and 5 ml sulphuric acid were added successively. The procedure for sample digestion was similar to that for phosphorus estimation. The digested sample was cooled and diluted to 250 ml with distilled water in a volumetric flask.

Among the reagents, saturated ammonium oxalate solution was prepared by dissolving approximately 40 g ammonium oxalate in 1 l distilled water. Methyl red indicator was prepared by dissolving 0.125 g methyl red in ethanol; ammonium hydroxide was prepared by diluting one part concentrated ammonium hydroxide with four parts of distilled water; dilute sulphuric acid was prepared by diluting one part concentrated acid with four parts of distilled water; and 0.05 N potassium permanganate was prepared by dissolving 1.6 g potassium permanganate in 1 l boiling distilled water.

Fifty ml aliquot of the digested test sample was pipetted into a 250 ml beaker, heated to boiling, and 10 ml of hot ammonium oxalate solution and one drop of methyl red

indicator were added. Ammonium hydroxide was added drop by drop till solution became pink, followed by boiling for 2 min. The beaker was cooled and the colour of the solution was adjusted to faint pink by addition of ammonium hydroxide. After 4 – 5 h, the solution was filtered and the precipitate was washed 5 - 6 times with distilled water. The filter paper was pierced by means of a glass rod and the precipitate was washed into the beaker in which precipitation was made. Thereafter, the filter paper was washed alternately with hot water and hot dilute sulphuric acid. Finally, 20 ml of dilute sulphuric acid was added, and the beaker was heated at 80 °C before titrating with 0.05 N potassium permanganate (till pink colour just persisted). The percent calcium content in the sample was calculated as:

$$\begin{aligned} & \mathbf{1\ ml\ of\ 0.05\ N\ potassium\ permanganate = 1\ mg\ calcium;} \\ \mathbf{\% \ Calcium} & \mathbf{= (weight\ of\ calcium\ in\ aliquot,\ g) \times (aliquot\ factor)} \\ & \mathbf{\times (100/\ weight\ of\ sample,\ g)} \end{aligned}$$

Fats: Ten g of the powdered test sample was transferred into a thumble tube and placed in the extractor part of the Soxhlet apparatus. Thereafter, $\frac{3}{4}$ of the reservoir of the Soxhlet apparatus was filled with petroleum ether, the apparatus was fixed with the help of stands, and placed on a hot plate. The condenser of the Soxhlet apparatus was connected with a continuous water current with the help of rubber tube. The extraction process was continued for 4 - 6 h till all the fatty materials dissolved in the petroleum ether in the reservoir. Thereafter, the apparatus was removed, and the flask was placed in waterbath to evaporate the petroleum ether. The flask was cooled after complete evaporation of the petroleum ether, and the cooled flask along with lipids was weighed (A g). Thereafter, the flask was washed, dried and weighed in empty condition (B g). The difference between the two weights (A - B g) indicated the amount of lipids in the test sample.

3.8.3. Soil

Texture: Soil texture was determined by sieving and weighing method (Piper, 1944). Twenty-five g soil sample was passed through a series of sievings with sieves of different mesh sizes, and the soil retained by each sieve was weighed.

pH: Twenty g soil sample was transferred to 125 ml wide mouth bottle, to which 100 ml distilled water was added. The mouth of the bottle was fixed by a stopper, and the

bottle was shaken intermittently for 1 h. The pH of this solution was determined by potentiometric measurement as was the case with water pH (section 3.7.1).

Organic carbon: Walkley and Black's (1934) method was adopted for determining the organic carbon content of the soil samples. Five g soil sample was transferred to a dry 500 ml Erlenmeyer flask to which 10 ml of normal potassium dichromate solution and 20 ml concentrated sulphuric acid were added successively. The flask was shaken for 2 - 3 min and allowed to stand on asbestos sheet for 30 min. Thereafter, 200 ml distilled water, 10 ml of 85 % phosphoric acid and 1 ml of diphenylamine indicator were added into the flask. The mixture thus obtained was titrated against normal ferrous sulphate solution filled in a burette, until the colour of the mixture became green. Thereafter, 0.05 ml of normal potassium dichromate was further added to the flask, and the solution was again titrated against normal ferrous sulphate solution until the last traces of blue colour disappeared,

The percentage of soil carbon (C) was calculated as :

$$C (\%) = \frac{V1-V2}{W} \times 0.003 \times 100$$

where,

V1 = Volume of normal potassium dichromate (ml);

V2 = Volume of normal ferrous sulphate solution in ml;

W = Weight of the soil in g.

Percentage of organic matter was obtained by multiplying the percentage of organic carbon by the factor 1.724.

Nitrogen, phosphorus and potassium: The methods and procedures for the estimation of nitrogen, phosphorus and potassium in soil samples were similar to section 3.8.2 (*Spirodela*, fishmeals, and fish tissues) except that the sample weights were ten times greater than in the section.

3.9. STATISTICAL ANALYSES

The SPSS software was used: (i) to find out the Pearson correlation coefficient between *S. polyrhiza* (biomass and nutrients) and dissolved nutrients (nitrogen, phosphorus and potassium) in habitat water; (ii) to compare the mean values of different frequencies of duckweed manuring with control on height, number of leaves, leaf area and resource allocation pattern in brinjal and chilli crops, and (iii) to compare the impact of duckweed manurings with control on soil properties (organic matter, pH, nitrogen, phosphorus and potassium) under brinjal and chilli crops.

CHAPTER 4
RESULTS AND DISCUSSIONS

4. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. *IN – SITU* GROWTH

4.1.1.1. Seasonal Variations in Biomass and Nutrients

The range of some physico-chemical parameters of water in the pool harbouring *S. polyrhiza* colony was recorded as follows : temperature 17.2 (January) and 31.2 °C (September) ; pH 6.2 (January and September) and 6.4 (May) ; total dissolved solids 127.4 (January) and 650 mg/ l (September) ; nitrogen 3.3 (September) and 8.2 mg/ l (January) ; phosphorus 0.1 (May and September) and 0.5 mg/ l (January) ; and potassium 4.3 (September) and 19.7 mg/ l (January) (Table 4.1).

Comparatively, the highest fresh biomass (823 g/ m²) and nutrient concentration (protein 19.6 %, phosphorus 0.9 %, and potassium 2.1 %) in *S. polyrhiza* occurred in January (winter season) ; whereas the lowest biomass (397.2 g/ m²), proteins (15.5 %) and phosphorus (0.6 %) were recorded in May (summer season) (Table 4.2). The potassium concentration in the duckweed tissues was least (1.3 %) in September (rainy season).

Statistically, strong positive correlations existed between *S. polyrhiza* biomass and dissolved nutrients (nitrogen $r = 0.94$, $p < 0.01$; phosphorus $r = 0.97$, $p < 0.01$; potassium $r = 0.97$, $p < 0.01$) in habitat water. Similarly, protein ($r = 0.68$, $p < 0.05$), phosphorus ($r = 0.74$, $p < 0.05$), and potassium ($r = 0.71$, $p < 0.05$) contents in *S. polyrhiza* tissues were also positively correlated with dissolved levels of these nutrients in habitat water (Table 4.3).

Table 4.1. Seasonal variations in physico-chemical properties of water (mean \pm SE ; n = 3)

	January	May	September
Temperature (°C)	17.2 \pm 2.4	30.6 \pm 3.2	31.2 \pm 3.2
Ph	6.2 \pm 1.4	6.4 \pm 1.4	6.2 \pm 1.4
Total dissolved solids (mg/ l)	127.4 \pm 6.5	267.0 \pm 9.8	650.0 \pm 14.7
Nitrogen (mg/ l)	8.2 \pm 1.6	4.4 \pm 1.2	3.3 \pm 1.0
Phosphorus (mg/ l)	0.5 \pm 0.4	0.1 \pm 0.2	0.1 \pm 0.2
Potassium (mg/ l)	19.7 \pm 2.5	6.4 \pm 1.4	4.3 \pm 1.2

Table 4.2. Seasonal variations in fresh biomass (mean \pm SE ; n = 10) and nutritive value (mean \pm SE ; n = 3) of *S. polyrhiza*

	January	May	September
Biomass (g/ m ²)	823 \pm 9.1	397.2 \pm 6.3	453.5 \pm 6.7
Protein (%)	19.6 \pm 2.5	15.5 \pm 2.3	16.6 \pm 2.3
Phosphorus (%)	0.9 \pm 0.5	0.6 \pm 0.4	0.6 \pm 0.4
Potassium (%)	2.1 \pm 0.8	2.1 \pm 0.8	1.3 \pm 0.6

Table 4.3. Correlation analysis between *S. polyrhiza* (biomass and nutrients) and dissolved nutrients in habitat water

		Dissolved nutrients in water		
		Nitrogen (mg/l)	Phosphorus (mg/l)	Potassium (mg/l)
Biomass (g/m ²)	Pearson Correlation	0.939**	0.968**	0.969**
	Sig. (2-tailed)	0.000	0.000	0.000
	N	9	9	9
Protein (%)	Pearson Correlation	0.618	0.790*	0.658
	Sig. (2-tailed)	0.076	0.011	0.054
	N	9	9	9
Phosphorus (%)	Pearson Correlation	0.651	0.743*	0.706*
	Sig. (2-tailed)	0.058	0.022	0.034
	N	9	9	9
Potassium (%)	Pearson Correlation	0.386	0.342	0.347
	Sig. (2-tailed)	0.305	0.367	0.361
	N	9	9	9

* Correlation significant at p < 0.05 level (2-tailed)

** Correlation significant at p < 0.01 level (2-tailed)

4.1.2. Morphological Variations

The *S. polyrhiza* fronds were largest (72.06 mm²) in size in April and smallest (29.19 mm²) in February in water body 1 ; whereas largest (43.17 mm²) and smallest (28.85 mm²) fronds occurred in February and December, respectively in water body 2 ; and in February (41.07 mm²) and December (24.1 mm²), respectively in water body 3 (Table 4.4)

Root length of *S. polyrhiza* was minimum (0.58 cm) in May and maximum (1.17 cm) in March in water body 1, between 0.68 cm (December) and 1.79 cm (February) in water body 2, and between 0.78 cm (April) and 2.48 cm (December) in water body 3 (Table 4.4).

Number of fronds per unit area ranged between 3700/ m² (February) and 50866/ m² (January) in water body 1, between 20300/ m² (April) and 64067/ m² (March) in water body 2, and between 38767/ m² (May) and 59100/ m² (January) in water body 3 (Table 4.4).

The fresh weight of *S. polyrhiza* fronds ranged between 7.97 g/ m² (February) and 669.02 g/ m² (April) in water body 1, between 21.76 g/ m² (February) and 243.3 g/ m² (May) in water body 2, and between 95.19 g/ m² (March) and 380.52 g/ m² (January) in water body 3 ; whereas dry weight of fronds ranged between 0.35 g/ m² (February) and 53 g/ m² (April) in water body 1, between 5.03 g/ m² (February) and 21.06 g/ m² (May) in water body 2, and between 8.88 g/ m² (March) and 38.22 g/ m² (February) in water body 3 (Table 4.4).

The range of pH for water samples was 6.2 (December, February, March, May) and 6.4 (January and April) in water body 1, between 5.8 (January) and 6.3 (December, March and May) in water body 2, and between 5.8 (January) and 6.2 (April) in water body 3 ; whereas water temperature ranged between 18.1 (January) and 28.4°C (May) in water body 1, between 17 (January) and 27.5 °C (May) in water body 2, and between 16.5 (February) and 27.7 °C (May) in water body 3 (Table 4.4).

Water samples from all the selected water bodies had higher concentrations of nitrogen, phosphorus and potassium in January than May (Table 4.5). The nitrogen

concentration in water ranged between 3.37 and 5.25 mg/ l in water body 1, between 3.37 and 4.74 mg/ l in water body 2, and between 0.98 and 2.46 mg/ l in water body 3 ; phosphorus concentration ranged between 0.07 and 0.11 mg/ l in water body 1, between 0.05 and 0.12 mg/ l in water body 2, and between 0.12 and 0.19 mg/ l in water body 3 ; whereas potassium concentration in water ranged between 6.4 and 19.7 mg/ l in water body 1, between 4.57 and 4.99 mg/ l in water body 2, and between 7.65 and 13.2 mg/ l in water body 3 (Table 4.5).

TABLE 4.4 Monthly variations in some morphological attributes of *Spirodela polyrhiza* in selected water bodies (mean \pm SE; n = 3)

	Dec		Jan		Feb		Mar		Apr		May	
Water body 1												
Water temperature (°C)	19.20	\pm 2.50	18.10	\pm 2.40	18.50	\pm 2.50	23.00	\pm 2.80	26.70	\pm 3.00	28.40	\pm 3.10
Water Ph	6.20		6.40		6.20		6.20		6.40		6.20	
FronD size (mm²)	42.80	\pm 3.77	31.11	\pm 3.22	29.19	\pm 3.11	61.11	\pm 4.51	72.06	\pm 4.9	43.69	\pm 3.81
FronD number/ m²	46200.00	\pm 124.00	50866.00	\pm 130.00	3700.00	\pm 35.00	13533.00	\pm 67.00	40533.00	\pm 116.00	28940.00	\pm 98.00
FronD fresh weight (g/ m²)	610.75	\pm 14.26	425.50	\pm 11.90	7.97	\pm 1.62	101.88	\pm 5.82	669.02	\pm 14.93	427.31	\pm 11.90
FronD dry weight (g/ m²)	22.56	\pm 2.74	17.58	\pm 2.42	0.35	\pm 0.34	11.39	\pm 1.94	53.00	\pm 4.20	21.87	\pm 2.70
Root length (cm)	0.80	\pm 0.51	0.89	\pm 0.54	0.75	\pm 0.50	1.17	\pm 0.62	1.09	\pm 0.60	0.58	\pm 0.43

Contd.....

Water body 2

Water temperature (°C)	19.00 ± 2.50	17.00 ± 2.40	18.80 ± 2.50	22.90 ± 2.80	26.70 ± 3.00	27.50 ± 3.00
Water Ph	6.30	5.80	6.00	6.30	6.20	6.30
FronD size (mm²)	28.85 ± 3.10	30.65 ± 3.19	43.17 ± 3.79	27.37 ± 3.02	31.42 ± 3.23	28.42 ± 3.07
FronD number/ m²	21633.00 ± 84.00	39438.00 ± 114.00	15726.00 ± 72.00	64067.00 ± 146.00	20300.00 ± 82.00	36167.00 ± 109.00
FronD fresh weight (g/ m²)	87.32 ± 5.39	108.34 ± 6.01	21.76 ± 2.69	168.69 ± 7.49	107.08 ± 5.97	243.30 ± 9.005
FronD dry weight (g/ m²)	12.25 ± 2.02	12.96 ± 2.07	5.03 ± 1.29	9.35 ± 1.76	9.00 ± 1.73	21.06 ± 2.64
Root length (cm)	0.68 ± 0.47	1.27 ± 0.65	1.79 ± 0.77	1.50 ± 0.70	1.67 ± 0.74	1.52 ± 0.71

Contd.....

Water body 3

Water temperature (°C)	17.40 ± 2.40	17.20 ± 2.40	16.50 ± 2.30	20.70 ± 2.80	26.70 ± 3.00	27.50 ± 3.00
Water Ph	6.20	5.80	6.20	6.20	6.30	6.20
FronD size (mm²)	24.10 ± 2.83	24.98 ± 2.88	41.07 ± 3.70	34.25 ± 3.37	34.41 ± 3.38	36.11 ± 3.46
FronD number/ m²	49700.00 ± 128.00	59100.00 ± 140.00	32800.00 ± 104.00	18400.00 ± 78.00	48700.00 ± 127.00	38767.00 ± 113.00
FronD fresh weight (g/ m²)	344.32 ± 10.70	380.52 ± 11.26	247.30 ± 9.07	95.19 ± 5.63	340.12 ± 10.64	269.11 ± 9.47
FronD dry weight (g/ m²)	36.45 ± 3.48	38.22 ± 3.56	14.39 ± 2.19	8.88 ± 1.72	16.04 ± 2.31	21.00 ± 2.64
Root length (cm)	2.48 ± 0.90	1.66 ± 0.74	1.87 ± 0.78	1.73 ± 0.75	0.78 ± 0.50	0.93 ± 0.55

Table 4.5 Nutrient concentration in the selected water bodies (mean \pm SE ; n = 3)

	Water body 1		Water body 2		Water body 3	
	Jan	May	Jan	May	Jan	May
Nitrogen (mg/ l)	5.25 \pm 1.32	3.37 \pm 1.05	4.74 \pm 1.25	3.37 \pm 1.05	2.46 \pm 0.90	0.98 \pm 0.57
Phosphorus (mg/ l)	0.11 \pm 0.17	0.07 \pm 0.15	0.12 \pm 0.20	0.05 \pm 0.12	0.19 \pm 0.24	0.12 \pm 0.20
Potassium (mg/ l)	19.70 \pm 2.56	6.40 \pm 1.45	4.99 \pm 1.28	4.57 \pm 1.23	13.20 \pm 2.09	7.65 \pm 1.59

4.1.3. GROWTH ON DIFFERENT WATER pH AND NUTRIENT MEDIA

4.1.3.1. Different Water pH

S. polyrhiza tolerated the pH range of water only between 4 - 7 when incubated alone, and optimum growth occurred at pH 6 (Table 4.6). However, when incubated in mixture with *Lemna aequinoctialis*, *S. polyrhiza* survived in 2 - 10 range of water pH although gain in fresh weight at pH 6 was comparatively less than in pure culture.

Table 4.6 Increase/ decrease in fresh weight (g) of *S. polyrhiza* (mean \pm SE; n = 3) after incubation (either alone or in mixture with *Lemna aequinoctialis*) on different water pH regimes for 30 days. Figures in parentheses represent the fresh weight of inoculum.

Water pH	<i>Spirodela polyrhiza</i> (1 g)	<i>Spirodela polyrhiza</i> + (1 g)	<i>Lemna aequinoctialis</i> (1 g)
2	-	0.60 \pm 0.24	0.64 \pm 0.25
3	-	0.79 \pm 0.28	0.92 \pm 0.30
4	0.80 \pm 0.28	1.08 \pm 0.33	1.80 \pm 0.42
5	2.38 \pm 0.49	1.15 \pm 0.34	2.01 \pm 0.45
6	4.15 \pm 0.64	1.34 \pm 0.37	2.17 \pm 0.46
7	2.50 \pm 0.50	1.32 \pm 0.36	1.96 \pm 0.44
8	-	1.00 \pm 0.32	1.40 \pm 0.37
9	-	0.60 \pm 0.24	1.29 \pm 0.36
10	-	0.38 \pm 0.19	0.81 \pm 0.28

4.1.3.2. Inorganic Fertilizer

The best concentration of inorganic fertilizer per l of borewell water was N 10 mg, P 2 mg and K 2 mg for the growth of *S. polyrhiza*. At this concentration, frond fresh weight increased by 59 % than control after a fortnight incubation (Fig. 4.1). Higher concentrations of inorganic fertilizer had detrimental effect on frond growth.

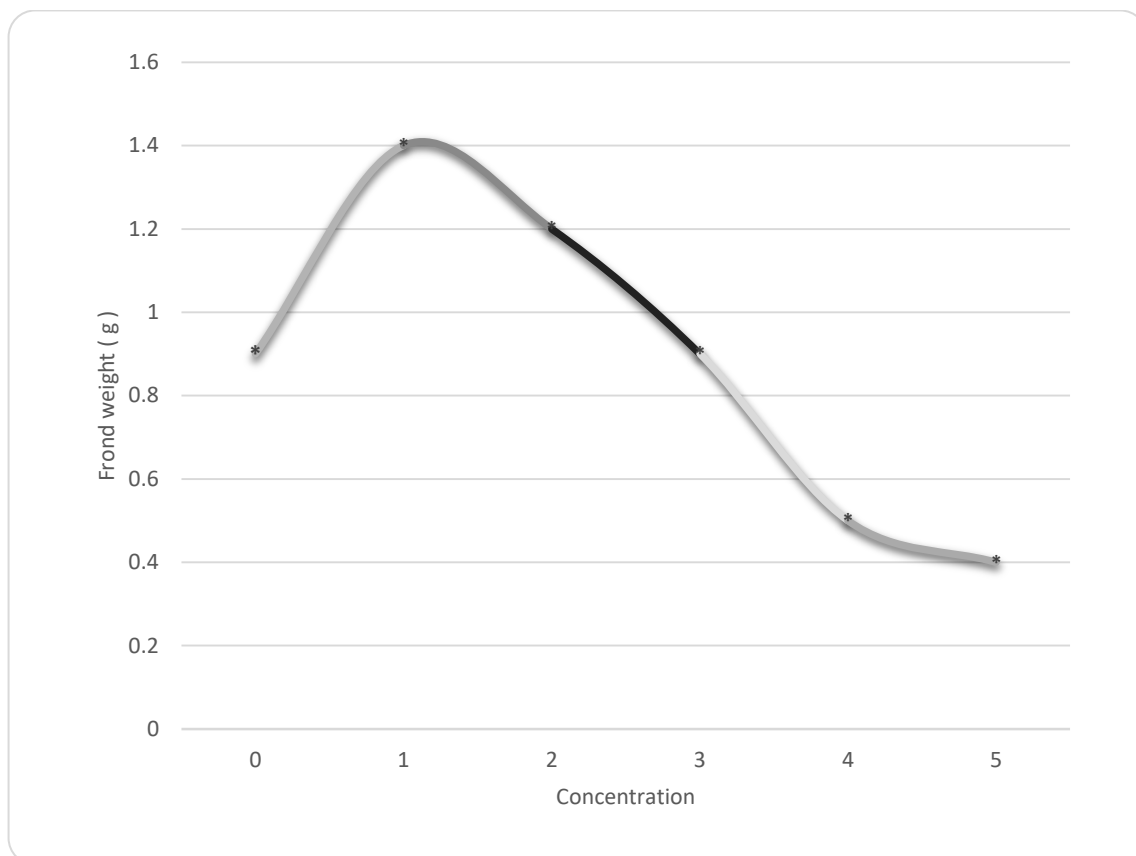


Figure 4.1 Increase/ decrease in fresh weight (1 g) of *S. polyrhiza* fronds after incubation on different concentrations of NPK fertilizer per l of water for a fortnight; 0 = Control ; 1 = N : P : K :: 10 mg : 2 mg : 2 mg; 2 = N : P : K :: 20 mg : 4 mg : 4 mg; 3 = N : P : K :: 30 mg : 6 mg : 6 mg; 4 = N : P : K :: 40 mg : 8 mg : 8 mg; 5 = N : P : K :: 50 mg : 10 mg : 10 mg

4.1.3.3. Sugars

Although appropriate concentration of glucose, sucrose and xylose in borewell water for the growth of *S. polyrhiza* was 6 g/l, 2 g/l and 6 g/l, respectively; comparatively, the best growth was observed on 2 g/l sucrose concentration in the present study (Fig. 4.2).

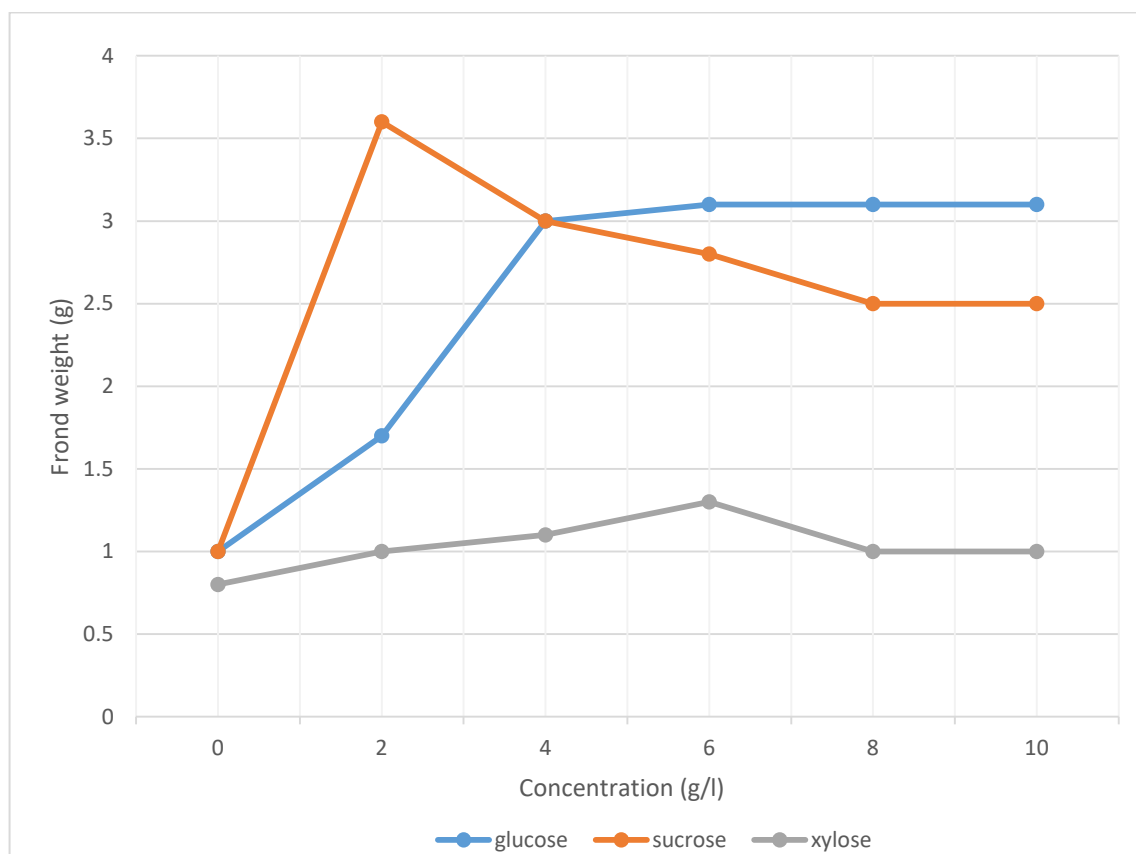


Figure 4.2 Increase/ decrease in fresh weight (1 g) of *S. polyrhiza* fronds after incubation on different concentrations of glucose, sucrose, and xylose

4.1.4. TURION FORMATION

After transference from habitat to borewell water, each *S. polyrhiza* frond produced 2 - 4 daughter fronds within 10 days. Thereafter, production of new fronds stopped, and turions became visible in the axils of daughter fronds (Plate 4) on the 16th day of transference of mother fronds to borewell water. The specific turion yield (i.e., the number of turions formed per *S. polyrhiza* frond) on the 22nd day of incubation of fronds on borewell water (at that time most of the fronds were decaying) was 3.2 ; and average length, width and weight of the dark-green turions were 4 mm, 3 mm, and 0.8 mg, respectively (Plate 4). On the other hand, fronds incubated

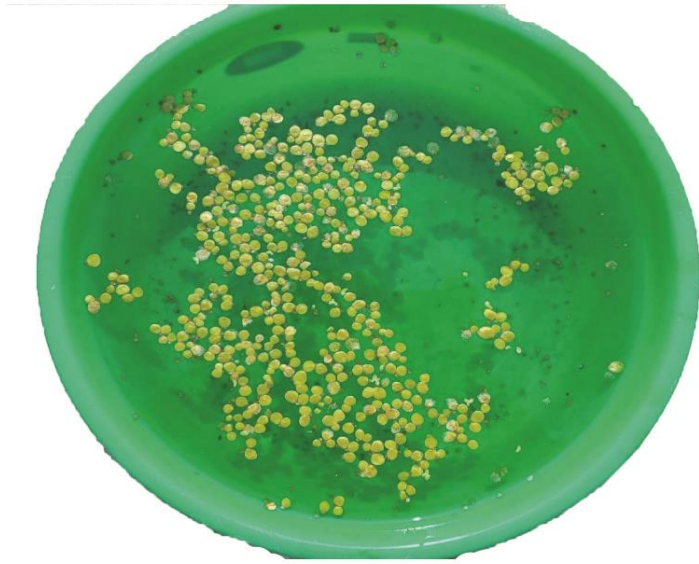


Plate 4 Turion forming fronds (top) and turions (bottom) of *Spirodela polyrhiza*

simultaneously on habitat water even for 22 days did not form any turion. The average minimum and maximum atmospheric temperatures during the incubation period of fronds on borewell as well as habitat water for turion formation were 6.8 and 27.6 °C, respectively.

A comparison of some physico-chemical parameters of habitat water of *S. polyrhiza* and borewell water is given in Table 4.7. Although pH and phosphorus concentration were slightly higher, the nitrogen and potassium concentrations were far more in the habitat water than the borewell water.

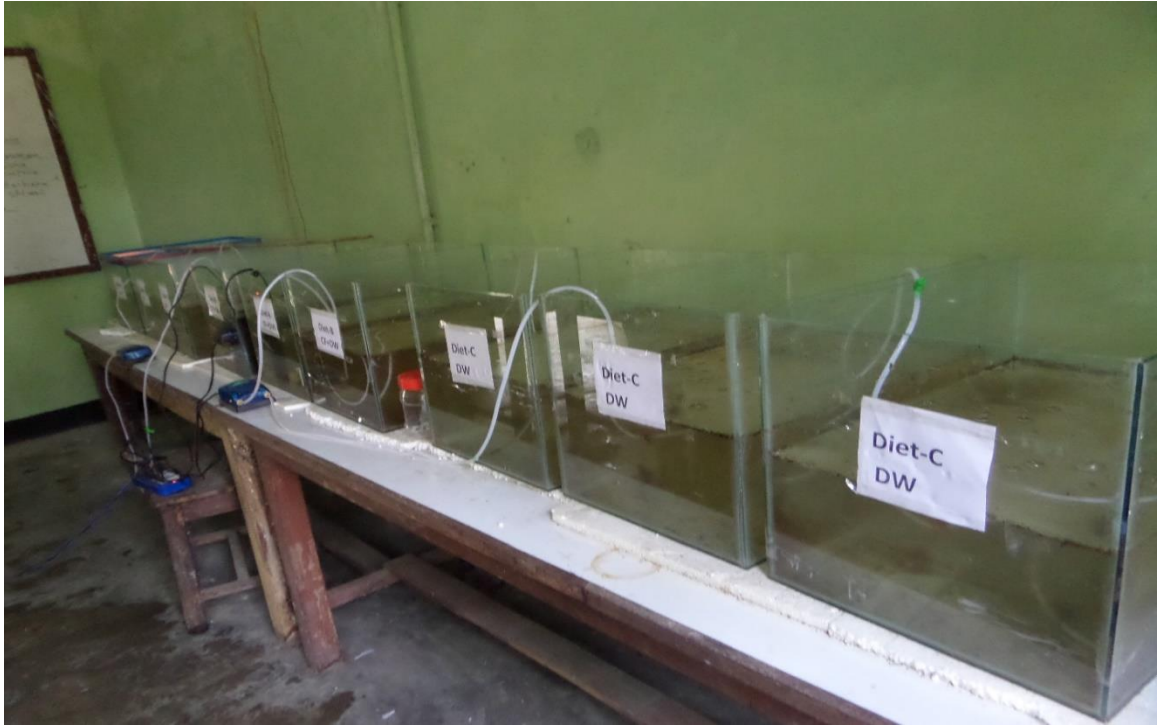
Table 4.7 Comparison of some physico-chemical properties of habitat water of *S. polyrhiza* and borewell water (mean \pm SE ; n = 3)

	pH	Nitrogen (mg/l)	Phosphorus (mg/ l)	Potassium (mg/ l)
Borewell water	6.4	0.20 \pm 0.26	< 0.05 \pm 0.13	3.26 \pm 1.04
Habitat water	7.1	4.30 \pm 1.19	0.06 \pm 0.14	15.50 \pm 2.27

4.1.5. FISHMEAL REPLACER

Tilapia (*Oreochromis niloticus* L.) fingerlings reared solely on conventional feed (CF) for 21 days had the highest increment (65 %) in weight in comparison to those reared on the mixture of CF and *S. polyrhiza* powder (GD) (37 %) or GD alone (9 %) (Table 4.8). Further, the tilapia reared on CF alone were golden - white, whereas those reared on the mixture of CF and GD, or GD alone were white and blackish in appearance, respectively (Plate 5). Among the feeds used, CF had higher concentration of fats although total ash and calcium contents were higher in GD. Protein and phosphorus contents were slightly more in CF in comparison to the GD (Table 4.9).

Nutritionally, tilapia reared on the mixture of CF and GD contained more proteins (50.3 %) than those reared on CF (45.34 %) or GD (45.79 %) alone (Table 4.10). Nevertheless, calcium and phosphorus concentrations were more in fish reared on the CF in comparison to those reared on the mixture of CF and GD as well as those reared solely on GD.



CF

CF + DW

DW

Plate 5 Culture of Nile tilapia on different feeds (top), and colour changes in the fish reared on different feeds (bottom) (CF = conventional feed; DW = GD/ duckweed meal)

Table 4.8 Morphological variations in Nile tilapia reared on different feeds for 21 days (mean \pm SE ; n = 3)

	Appearance	Length (cm)	Breadth (cm)	Weight (g)	Growth increment (%)
Conventional feed (CF)	Golden-white	11.23 \pm 1.93	3.73 \pm 1.11	22.19 \pm 2.72	65 \pm 5
<i>S. polyrhiza</i> powder (GD)	Blackish	10.10 \pm 1.83	3.73 \pm 1.06	14.64 \pm 2.21	09 \pm 2
CF + GD	White	10.23 \pm 1.84	3.16 \pm 1.10	18.40 \pm 2.47	37 \pm 4

Table 4.9 Nutrients (%) in conventional feed (CF) and *S. polyrhiza* powder (mean \pm SE; n = 3)

	Fats	Proteins	Total ash	Calcium	Phosphorus
GD	1.56 \pm 0.72	19.21 \pm 2.52	22.99 \pm 2.76	0.84 \pm 0.53	0.27 \pm 0.30
CF	7.89 \pm 1.62	22.98 \pm 2.76	4.27 \pm 1.19	0.13 \pm 0.20	0.40 \pm 0.36

Table 4.10 Nutrient accumulation (%) in tilapia reared on different feeds (mean \pm SE; n = 3)

	Fats	Proteins	Ash	Calcium	Phosphorus
CF	10.09 \pm 1.83	45.34 \pm 3.88	20.25 \pm 2.60	0.56 \pm 0.43	0.39 \pm 0.36
CF + GD	11.32 \pm 1.94	50.30 \pm 4.09	19.24 \pm 2.53	0.42 \pm 0.37	0.24 \pm 0.28
GD	11.44 \pm 1.95	45.79 \pm 3.91	18.24 \pm 2.46	0.21 \pm 0.26	0.31 \pm 0.32

4.1.6. WASTEWATER TREATMENT

4.1.6.1. Removal of Nutrients

The fresh weight of *S. polyrhiza* fronds increased from initial 1 g to 3.5 g after incubation on wastewater for 30 days. Further, culture of *S. polyrhiza* for 30 days reduced the concentration of major nutrients in wastewater in the order: nitrogen (25 %) > potassium (24 %) > phosphorus (22 %) (Table 4.11).

Table 4.11 Improvement in water quality induced by *S. polyrhiza* culture (mean \pm SE; n = 3)

	Initial water quality	Changes in quality 30 days after culture
Ph	7.4	7.2
Nitrogen (mg/l)	6.9 \pm 1.5	5.2 \pm 1.3
Phosphorus (mg/l)	1.8 \pm 0.8	1.4 \pm 0.7
Potassium (mg/l)	11.5 \pm 2.0	8.7 \pm 1.7
<i>S. polyrhiza</i> biomass (g)	1.0 \pm 0.6	3.5 \pm 1.1

4.1.6.2. Response to Mercury Stress

The biomass of *S. polyrhiza* increased by 43.8 % after incubation on 2 mg/ l concentration of mercuric chloride for a week, whereas biomass increase was only 20.2 % in control in the same period (Table 4.12). Further, chlorophyll a, chlorophyll b and carotenoid contents in the duckweed incubated on 2 mg/ l concentration of mercuric chloride for a week were higher than control by 16.4, 29.6 and 59.1 %, respectively. Higher concentrations (> 2 mg/ l) of mercuric chloride had detrimental effects on both biomass and photosynthetic pigments, and the duckweed perished completely within a week on 8 mg/ l concentration of the mercuric chloride.

Table 4.12 Response of *S. polyrhiza* (5 g inoculum) to mercury stress (mean \pm SE ; n = 3)

Mercuric chloride (mg/l)	Biomass (g)	Chlorophyll a (mg/ g)	Chlorophyll b (mg/ g)	Carotenoids
0 (Control)	6.01 \pm 1.41	0.50 \pm 0.40	0.27 \pm 0.30	0.22 \pm 0.26
2	7.19 \pm 1.54	0.82 \pm 0.51	0.35 \pm 0.33	0.35 \pm 0.33
4	3.24 \pm 1.03	0.32 \pm 0.31	0.19 \pm 0.24	0.14 \pm 0.20
6	1.80 \pm 0.77	0.25 \pm 0.28	0.17 \pm 0.22	0.12 \pm 0.20
8	0.00	0.00	0.00	0.00

4.1.7. DUCKWEED MANURE

4.1.7.1. Impacts on Vegetable Crops

Height: In both manure - treated and untreated (Control) brinjal plants, height increased slowly up to 88 days after plantation (DAP), and thereafter increment in height was rapid until 116 DAP (Table 4.13). At 116 DAP, plants under 7 - D manure treatment had 31 cm height followed by those with 14 - D manure treatment (27.25 cm). Plants under 21 - D manuring (17.5 cm) and control (16.62 cm) were approximately similar in height at 116 DAP. Likewise, chilli plants were tallest (32.37 cm) under 7-D than other frequencies of manuring and control from 46 to 102 DAP (Table 4.14).

Number of leaves: The number of leaves per brinjal plant was few and approximately similar under all the manuring frequencies and control till 74 DAP, but at 134 DAP, number of leaves per plant occurred in the order: 82 (7 - D) > 59 (14 - D) > 45 (21 - D) > 38 (C) (Table 4.15). Similarly, in chilli also there was no remarkable difference in number of leaves per plant under different frequencies of manuring and control till 60 DAP, but at 134 DAP, the number of leaves per plant occurred in the order : 343 (7-D) > 223 (14-D) > 198 (21-D) > 151 (C) (Table 4.16).

Leaf area: Brinjal plants under different frequencies of manuring had more leaf area than control from 46 DAP onwards and, at 134 DAP, the leaf area per plant was recorded in the order : 2982 cm² (7-D) > 1921 cm² (14-D) > 1636 cm² (21-D) > 1432 cm² (C) (Table 4.17). Similarly, the leaf area of chilli plants under different frequencies of duckweed manuring was more than control from 74 DAP onwards and, at 134 DAP, the leaf area per plant occurred in the order : 1531 cm² (7-D) > 1498 cm² (14-D) > 925 cm² (21-D) > 700 cm² (C) (Table 4.18).

TABLE 4.13 Effect of different frequencies of duckweed manuring on the height (cm) of brinjal plants (mean \pm SE; n = 10)

DAP (Days after plantation)	C	7-D	14-D	21-D
46	2.80 \pm 0.52	3.96 \pm 0.63	3.26 \pm 0.57	2.16 \pm 0.46
53	3.06 \pm 0.55	4.11 \pm 0.64	3.36 \pm 0.58	2.71 \pm 0.52
60	3.11 \pm 0.56	4.25 \pm 0.65	3.38 \pm 0.58	2.83 \pm 0.53
67	3.50 \pm 0.59	5.13 \pm 0.72	4.66 \pm 0.68	2.91 \pm 0.54
74	4.25 \pm 0.65	5.33 \pm 0.73	4.75 \pm 0.69	3.83 \pm 0.62
81	5.38 \pm 0.73	7.91 \pm 0.89	6.25 \pm 0.79	5.06 \pm 0.71
88	6.50 \pm 0.80	9.41 \pm 0.97	7.25 \pm 0.85	5.66 \pm 0.75
95	8.83 \pm 0.94	11.66 \pm 1.08	10.50 \pm 1.02	6.90 \pm 0.83
102	9.08 \pm 0.95	16.05 \pm 1.27	13.75 \pm 1.17	8.66 \pm 0.93
109	14.12 \pm 1.19	23.00 \pm 1.51	20.50 \pm 1.43	14.00 \pm 1.18
116	16.62 \pm 1.29 a	31.00 \pm 1.76 b	27.25 \pm 1.65 a	17.50 \pm 1.32 a

*Means followed by the same letter are not significantly ($p < 0.05$) different using DMRT

TABLE 4.14 Effect of different frequencies of duckweed manuring on the height (cm) of chilli plants (mean \pm SE; n = 10)

DAP	C	7-D	14-D	21-D
46	12.41 \pm 1.11	13.00 \pm 1.14	8.00 \pm 0.89	12.21 \pm 1.10
53	13.10 \pm 1.14	13.65 \pm 1.68	8.45 \pm 0.92	12.68 \pm 1.13
60	13.78 \pm 1.17	14.41 \pm 1.20	8.95 \pm 0.95	13.00 \pm 1.14
67	15.70 \pm 1.25	16.00 \pm 1.26	10.43 \pm 1.02	13.58 \pm 1.17
74	16.25 \pm 1.27	16.98 \pm 1.30	11.90 \pm 1.09	15.98 \pm 1.26
81	17.76 \pm 1.33	18.50 \pm 1.36	13.60 \pm 1.17	16.91 \pm 1.30
88	18.16 \pm 1.34	20.08 \pm 1.42	14.83 \pm 1.21	17.90 \pm 1.33
95	19.00 \pm 1.38	20.75 \pm 1.44	14.96 \pm 1.22	17.93 \pm 1.34
102	20.08 \pm 1.42	22.58 \pm 1.50	16.75 \pm 1.29	20.33 \pm 1.43
109	25.62 \pm 1.60	25.37 \pm 1.59	23.00 \pm 1.52	25.00 \pm 1.58
116	26.81 \pm 1.64 a	32.37 \pm 1.80 b	27.62 \pm 1.66 a	26.09 \pm 1.61 a

*Means followed by the same letter are not significantly ($p < 0.05$) different using DMRT

TABLE 4.15 Effect of different frequencies of duckweed manuring on the number of leaves per brinjal plant (mean± SE; n=10)

DAP	C	7-D	14-D	21-D
46	3 ± 0.55	3 ± 0.55	3 ± 0.55	3±0.55
53	3 ± 0.55	3 ± 0.55	3 ± 0.55	3 ± 0.55
60	3 ± 0.55	4 ± 0.63	3 ± 0.55	3 ± 0.55
67	3 ± 0.55	4 ± 0.63	4 ± 0.63	3 ± 0.55
74	4 ± 0.63	5 ± 0.70	4 ± 0.63	4 ± 0.63
81	5 ± 0.70	9 ± 0.95	6 ± 0.77	5 ± 0.70
88	7 ± 0.84	10 ± 1.00	7 ± 0.84	5 ± 0.70
95	9 ± 0.95	16 ± 1.26	12 ± 1.09	8 ± 0.89
102	10 ± 1.00	17 ± 1.30	15 ± 1.22	9 ± 0.95
109	20 ± 1.41	43 ± 2.07	33 ± 1.82	20 ± 1.41
116	21 ± 1.45	52 ± 2.28	43 ± 2.07	22 ± 1.48
134	38 ± 1.95 a	82 ± 2.86 b	59 ± 2.43 c	45 ± 2.12 a

*Means followed by the same letter are not significantly ($p < 0.05$) different using DMRT

TABLE 4.16 Effect of different frequencies of duckweed manuring on the number of leaves per chilli plant (mean \pm SE; n=10)

DAP	C	7-D	14-D	21-D
46	8 \pm 0.89	8 \pm 0.89	7 \pm 0.83	8 \pm 0.89
53	8 \pm 0.89	8 \pm 0.89	7 \pm 0.83	8 \pm 0.89
60	9 \pm 0.94	10 \pm 1.00	7 \pm 0.83	9 \pm 0.94
67	10 \pm 1.00	15 \pm 1.22	11 \pm 1.05	11 \pm 1.05
74	14 \pm 1.18	24 \pm 1.55	15 \pm 1.22	18 \pm 1.34
81	20 \pm 1.41	37 \pm 1.92	24 \pm 1.55	26 \pm 1.61
88	25 \pm 1.58	51 \pm 2.26	33 \pm 1.82	30 \pm 1.73
95	32 \pm 1.79	61 \pm 2.47	35 \pm 1.87	38 \pm 1.94
102	39 \pm 1.97	72 \pm 2.68	49 \pm 2.21	59 \pm 2.43
109	69 \pm 2.62	120 \pm 3.46	90 \pm 3.00	92 \pm 3.03
116	85 \pm 2.92	177 \pm 4.20	106 \pm 3.26	100 \pm 3.16
134	151 \pm 3.89 a	343 \pm 5.86 b	223 \pm 4.72 c	198 \pm 4.45 c

*Means followed by the same letter are not significantly ($p < 0.05$) different using DMRT

Branch number: At the time of harvest (134 DAP), number of branches per brinjal plant occurred in the order : 14 (7-D and 14-D) > 9 (21-D) > 8 (C) (Fig. 4.3); whereas number of branches per chilli plant occurred in the order : 36 (7-D) > 32 (21-D) > 28 (14- D) > 18 (C) (Fig. 4.3).

Fruit yield: In brinjal plants, number of fruits per individual was 15, 12, 10 and 7 under 14 – D, 7 – D, 21 – D, and C, respectively, and fresh weight per fruit ranged between 84 (14 – D and 21 – D) and 88 g (7 – D) (Fig. 4.4). On the other hand, number of fruits per chilli plant was 44, 32, 29 and 17, under 7 – D, 14 – D, 21 – D and C, respectively ; and fresh weight per fruit ranged between 1.32 g (C) and 1.42 g (14 – D) (Fig. 4.5).

TABLE 4.17 Effect of different frequencies of duckweed manuring on leaf area (cm²) per brinjal plant (mean ± SE; n = 10)

DAP	C	7-D	14-D	21-D
46	33.42 ± 1.83	62.64 ± 2.50	40.85 ± 2.02	35.98 ± 1.89
53	33.79 ± 1.84	66.13 ± 2.57	52.19 ± 2.28	37.70 ± 1.94
60	38.68 ± 1.97	66.96 ± 2.59	54.44 ± 2.33	37.92 ± 1.95
67	42.15 ± 2.05	71.77 ± 2.68	56.16 ± 2.37	39.99 ± 1.99
74	50.82 ± 2.25	92.19 ± 3.03	65.10 ± 2.55	54.17 ± 2.32
81	78.05 ± 2.79	185.38 ± 4.30	120.94 ± 3.48	69.68 ± 2.63
88	135.58 ± 3.68	263.96 ± 5.14	158.99 ± 3.99	79.33 ± 2.81
95	261.68 ± 5.11	539.12 ± 7.34	320.89 ± 5.66	159.93 ± 3.99
102	365.36 ± 6.04	757.08 ± 8.70	607.28 ± 7.79	267.49 ± 5.17
109	675.56 ± 8.21	1613.75 ± 12.70	1217.70 ± 11.03	577.96 ± 7.60
116	791.18 ± 8.89	2034.00 ± 14.26	1419.83 ± 11.91	847.94 ± 9.20
134	1431.65 ± 11.96 a	2981.81 ± 17.27 b	1920.93 ± 13.86 c	1636.36 ± 12.79 a

*Means followed by the same letter are not significantly (p < 0.05) different using DMRT

TABLE 4.18 Effect of different frequencies of duckweed manuring on leaf area (cm²) per chilli plant (mean \pm SE; n = 10)

DAP	C	7-D	14-D	21-D
46	37.41 \pm 1.93	47.17 \pm 2.17	34.93 \pm 1.87	36.36 \pm 1.87
53	37.45 \pm 1.94	49.37 \pm 2.22	37.03 \pm 1.92	50.24 \pm 2.24
60	39.15 \pm 1.98	49.40 \pm 2.22	39.66 \pm 1.99	57.39 \pm 2.39
67	43.89 \pm 2.09	64.12 \pm 2.53	40.53 \pm 2.01	62.72 \pm 2.50
74	50.18 \pm 2.24	83.48 \pm 2.89	54.50 \pm 2.33	79.16 \pm 2.81
81	66.31 \pm 2.57	133.76 \pm 3.66	82.15 \pm 2.87	101.04 \pm 3.18
88	78.59 \pm 2.88	187.08 \pm 4.33	117.87 \pm 3.43	103.96 \pm 3.22
95	105.82 \pm 3.25	230.57 \pm 4.80	144.74 \pm 3.80	122.41 \pm 3.50
102	139.04 \pm 3.73	327.08 \pm 5.72	221.97 \pm 4.71	242.84 \pm 4.92
109	290.01 \pm 5.38	632.18 \pm 7.95	506.78 \pm 7.11	423.86 \pm 6.51
116	393.98 \pm 6.27	795.69 \pm 8.92	712.70 \pm 8.44	467.86 \pm 6.84
134	699.89 \pm 8.36 a	1530.90 \pm 12.37 b	1497.88 \pm 12.24 b	924.66 \pm 9.61 c

*Means followed by the same letter are not significantly (p < 0.05) different using DMRT

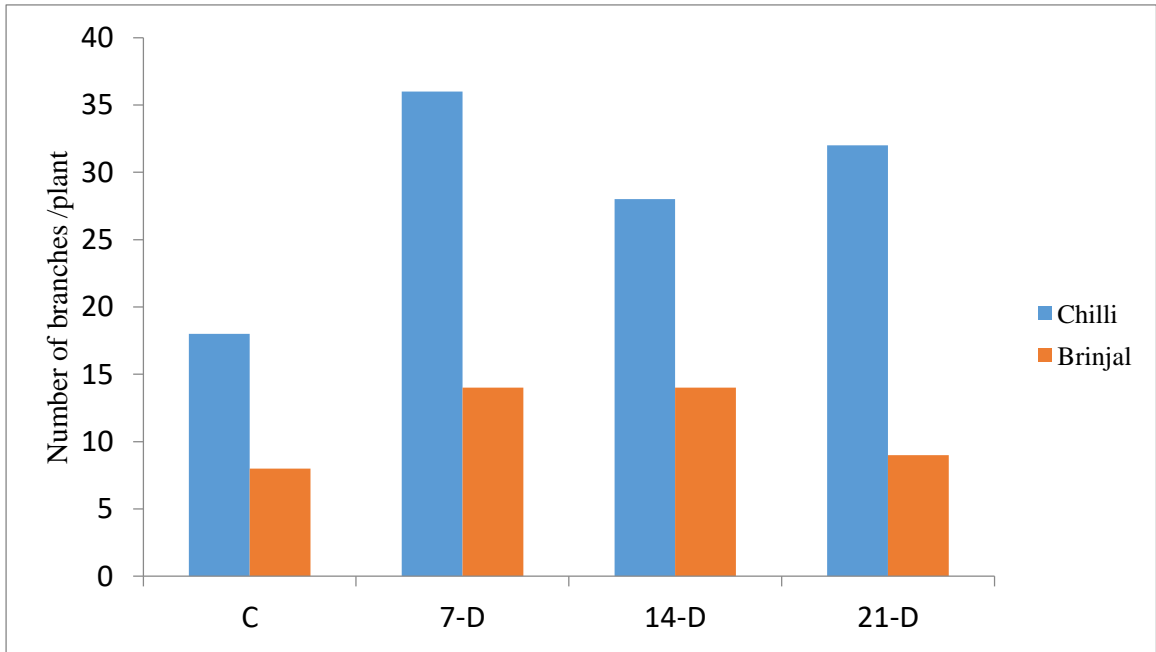


Fig. 4.3 Number of branches per plant at 134 DAP in the manure-treated and untreated individuals of the test species (n = 10)

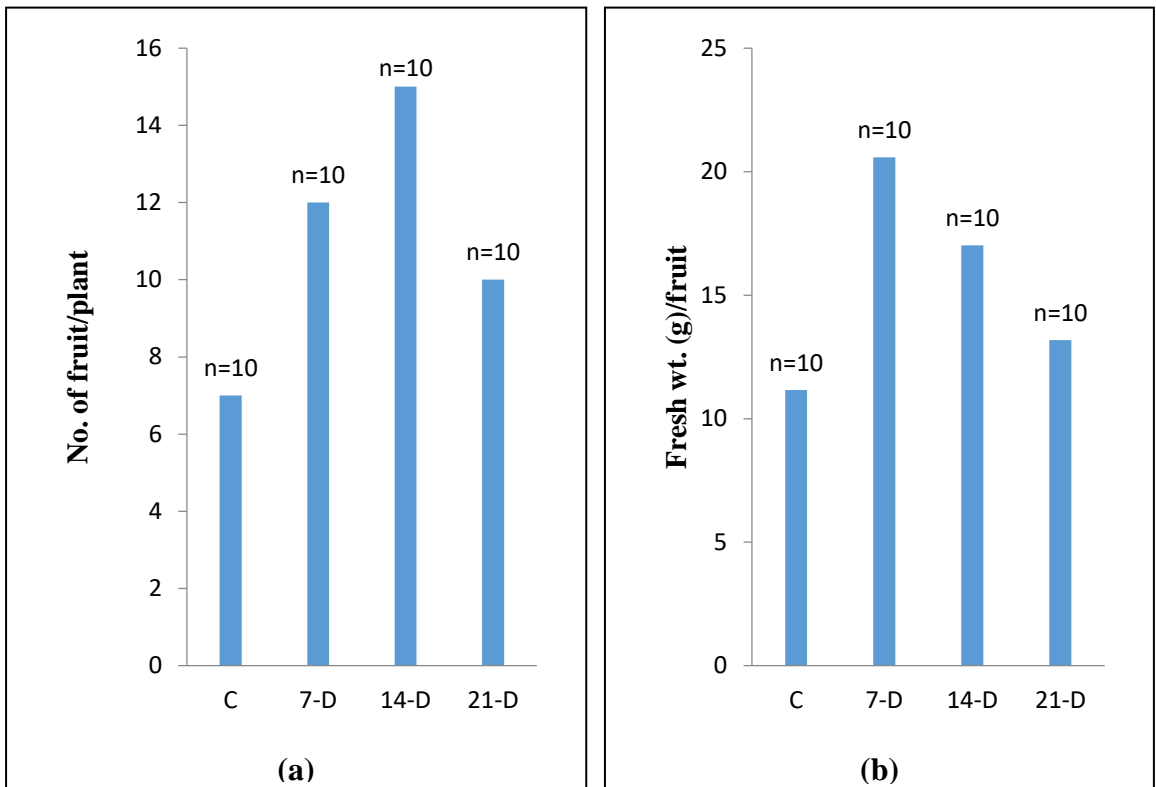


Fig 4.4 Effect of different frequencies of duckweed manuring on (a) fruit number/ plant and (b) fresh weight/fruit of brinjal plant

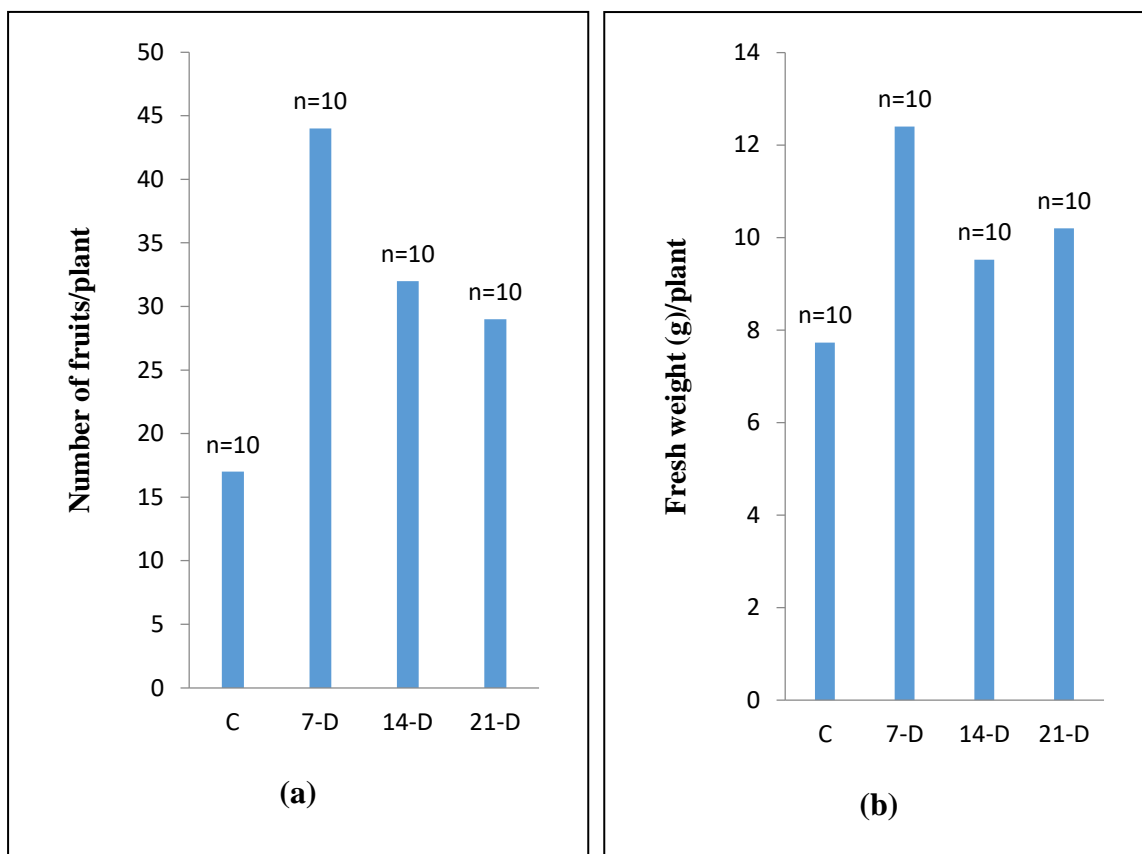


Fig 4.5 Effect of different frequencies of duckweed manuring on (a) fruit number/ plant, and (b) fresh weight/ fruit of chilli plants

Resource allocation: Total dry weight (g/plant) of brinjal plants under different frequencies of duckweed manuring and control occurred in the order : 20.58 g (7 – D) > 17.01 g (14 – D) > 13.18 g (21 – D) > 11.16 g (C) (Table 4.19). Roots had highest (30 %) contribution to total dry weight in 14 – D and 21 – D, and lowest (14 %) in 7 – D ; whereas stems had the highest (37 %) and the lowest (26 %) contribution to total dry weight in 7 – D and C, respectively (Fig. 4.6).

Total dry weight (g/plant) of chilli plants under different frequencies of manuring and control occurred in the order : 14.4 g (7 – D) > 10.2 g (21 – D) > 9.52 g (14 – D) > 7.13 g (C) (Table 4.19). Contribution of roots to total dry weight was highest (36 %) in control and lowest (22 %) in 7 – D (Fig. 4.7). Chilli plants had highest proportion of stems (43 %) in 7 – D and 14 – D, and lowest (29 %) under 21 – D. Contribution of leaves to total dry weight under different frequencies of manuring and control was recorded in the order: 40 % (21 – D) > 35 % (7 – D) > 32 % (14 – D) > 28 % (C).

Statistically, the number of leaves, leaf area, number of branches, fruit yield and total dry weight per plant were significantly ($p < 0.01$) much more than control under 7 – D and 14 – D manurings in both brinjal and chilli plants.

TABLE 4.19 Variations in dry weight of different parts (g/plant) of the test species under different frequencies of manure treatment and control (mean \pm SE; n = 10)

Test species	Plant parts	C	7-D	14-D	21-D
<i>Solanum melongena</i>	Leaves	5.74 \pm 0.75	10.04 \pm 1.00 **	6.94 \pm 0.83	5.39 \pm 0.73
	Stems	2.89 \pm 0.59	7.61 \pm 0.87 **	4.91 \pm 0.70 *	3.87 \pm 0.62
	Roots	2.53 \pm 0.50	2.93 \pm 0.54	5.16 \pm 0.71 *	3.92 \pm 0.63
	Total	11.16 \pm 1.06	20.58 \pm 1.43	17.01 \pm 1.30	13.18 \pm 1.14
<i>Capsicum annuum</i>	Leaves	2.15 \pm 0.46	4.38 \pm 0.66 *	3.07 \pm 0.55	4.15 \pm 0.64
	Stems	2.80 \pm 0.54	5.35 \pm 0.73 *	4.07 \pm 0.63	2.92 \pm 0.54
	Roots	2.78 \pm 0.52	2.67 \pm 0.51	2.38 \pm 0.48	3.13 \pm 0.56
	Total	7.13 \pm 0.84	12.40 \pm 1.11	9.52 \pm 0.92	10.20 \pm 1.00

*Means significantly different at $p < 0.05$ level; **Means significantly different at $p < 0.01$ level

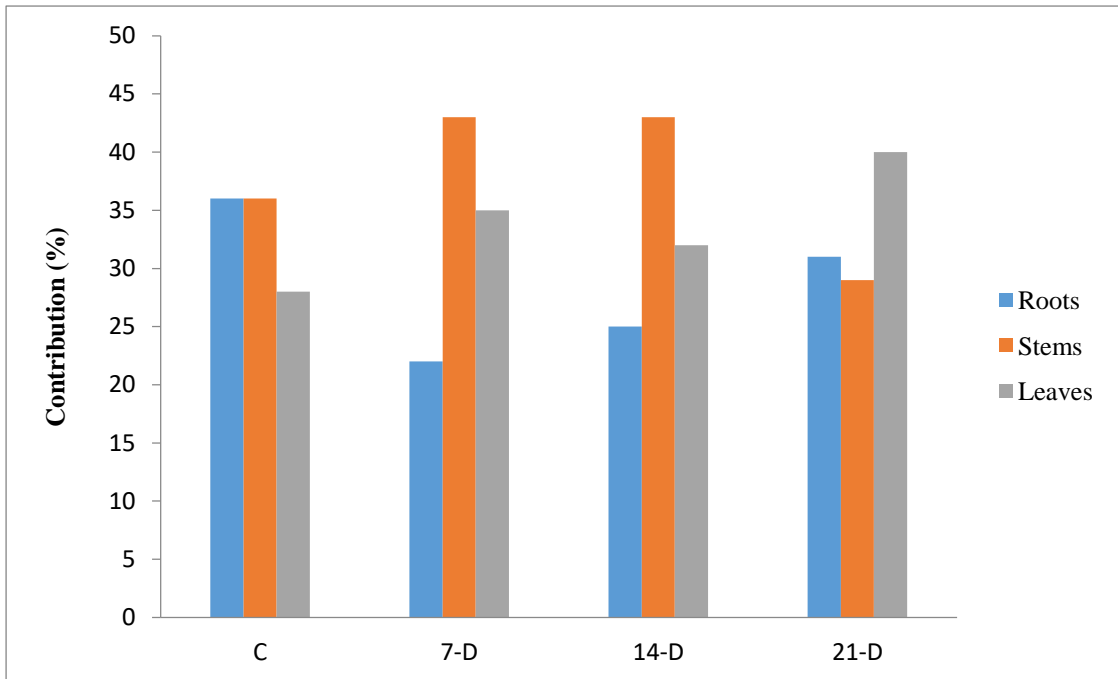


Fig 4.6 Contribution (%) of different parts to total dry weight per individual of manure-treated and untreated brinjal plants (n = 10)

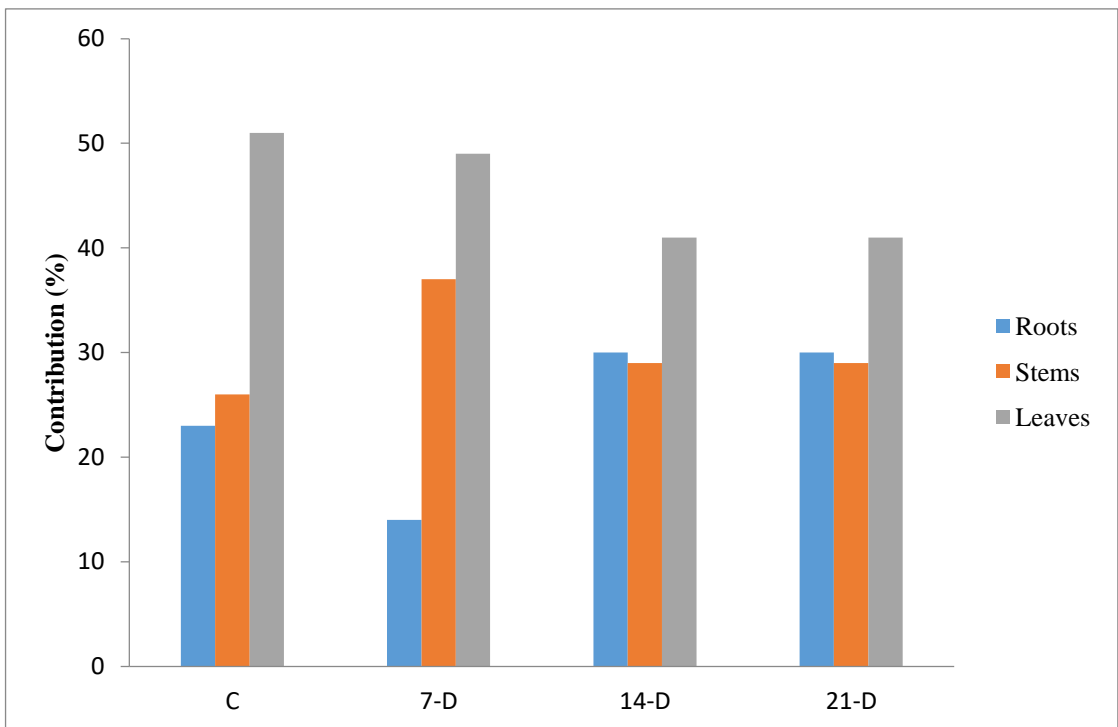


Fig 4.7 Contribution (%) of different parts to total dry weight per individual of manure-treated and untreated chilli plants



Plate 6 Effects of different frequencies of duckweed manuring on growth of brinjal crops after 102 days of plantation



Plate 7 Effects of different frequencies of duckweed manuring on growth of chilli crops after 102 days of plantation

4.1.7.2. Impacts on Soil Properties

The soil pH was slightly higher in pots planted with brinjal under 7 – D and 14 – D (6.6 and 6.3, respectively) than control, whereas pH of the soil was less than control (6.6) in chilli pots under all the frequencies of manuring (Table 4.20).

Soil samples had highest potassium concentration in control than manure-treated pots under cultivation of both the test species. The organic matter content of the soil was recorded in the order : 6.43 % (7 – D) > 5.96 % (C – D) > 5.17 % (14 – D) > 4.78 % (21 – D) under brinjal cultivation, whereas the order of organic matter content of soil samples under chilli cultivation was : 7.91 % (14 – D) > 6.82 % (7 – D) > 5.41 % (21 – D) > 3.84 % (C). The nitrogen content in soil samples under brinjal cultivation occurred in the order : 0.32 % (7 – D) > 0.30 % (C) > 0.26 % (14 – D) > 0.24 % (21 – D), whereas under chilli cultivation soil nitrogen occurred in the order : 0.4 % (14 – D) > 0.34 % (7 – D) > 0.27 % (21 – D) > 0.19 % (C).

Statistically, phosphorus concentration in soil under all the frequencies of duckweed manuring was astoundingly huge ($p < 0.01$) than control at the time of harvest (134 DAP) of both brinjal and chilli plants, whereas potassium concentration decreased significantly ($p < 0.01$) than control in soil under 21 – D duckweed manuring at the time of harvest of both the test species (Table 4.20).

TABLE 4.20 Effect of different frequencies of duckweed manuring on physico-chemical properties of soil (mean \pm SE; n = 3)

Parameters	C	7-D	14-D	21-D
Organic matter (%)	5.96 \pm 1.40 a	6.43 \pm 1.46 a	5.17 \pm 1.31 a	4.78 \pm 1.26 a
Ph	6.2 \pm 1.43 a	6.6 \pm 1.48 a	6.3 \pm 1.45 a	6.2 \pm 1.43 a
Brinjal sets				
N (%)	0.30 \pm 0.31 a	0.32 \pm 0.32 a	0.26 \pm 0.29 a	0.24 \pm 0.28 a
P (ppm)	4.92 \pm 1.28 a	65.79 \pm 4.68 b	119.57 \pm 6.31c	118.57 \pm 6.28 c
K (ppm)	44.18 \pm 3.83 a	36.38 \pm 3.48 b	41.58 \pm 3.72a	5.19 \pm 1.31 c
Organic matter (%)	3.84 \pm 1.13 a	6.82 \pm 1.50 b	7.91 \pm 1.62 b	5.41 \pm 1.34 a
pH	6.6 \pm 1.48 a	6.2 \pm 1.42 a	6.0 \pm 1.41 a	6.0 \pm 1.41 a
Chilli sets				
N (%)	0.19 \pm 0.25 a	0.34 \pm 0.33 b	0.40 \pm 0.36 b	0.27 \pm 0.30 a
P (ppm)	4.42 \pm 1.21 a	118.41 \pm 6.27 b	74.58 \pm 4.98c	112.10 \pm 6.11b
K (ppm)	54.57 \pm 4.26 a	46.78 \pm 3.94 a	51.98 \pm 4.16 a	7.79 \pm 1.61 b

*Means followed by the same letter are not significantly ($p < 0.05$) different using DMRT

4.2. DISCUSSION

4.2.1. IN – SITU GROWTH

4.2.1.1. Variations in Biomass and Nutrients

In the present study, the highest fresh biomass (823 g/ m²) and nutrient concentration (protein 19.6 % , phosphorus 0.9 %) in *S. polyrhiza* occurred in January when concentrations of nitrogen (8.2 mg/ l) and phosphorus (0.5 mg/ l) were also highest in water ; whereas the lowest biomass (397.2 g/ m²), proteins (15.5 %) and phosphorus (0.6 %) concentrations in the test species were recorded in May when dissolved nitrogen and phosphorus in water were low in concentration (4.4 and 0.1 mg/ l, respectively). Hasan and Chakrabarti (2009) have opined that the nutrient content of duckweed is probably more dependent on the mineral concentrations of the growth medium than on the species or their geographic location. Water low in nutrients generally results in reduced nutritional content and slow growth in duckweeds.

4.2.1.2. Morphological Variations

The optimum range of some important physico-chemical factors of water for the growth of duckweeds is as follows: temperature 15 – 30 °C, pH 6.5 - 8, nitrogen 7 - 12 mg/ l, and phosphorus 4 - 8 mg/ l (Hasan and Chakrabarti, 2009). Thus, except temperature, range of the pH, nitrogen and phosphorus in the selected pools were inadequate for the optimum growth of *S. polyrhiza*. Nevertheless, concentrations of nitrogen and phosphorus ranged between 3.37 - 5.25 mg/ l (0.98 - 2.46 mg/ l in water body 3) and 0.05 – 0.19 mg/ l, respectively, in the selected pools. Edwards *et al.* (1992) observed that pond water with less than 3 mg/ l N and 0.3 mg/ l P did not support normal growth of *Lemna perpusilla* and *S. polyrhiza*, whereas Luond (1980) reported growth reduction in duckweeds only when P concentration in water dropped below 0.017 mg/ l.

The smallest sized fronds of *S. polyrhiza* occurred in February, March and December in water body 1, 2 and 3, respectively; whereas fronds of the largest size were observed in April in water body 1, and in February in water body 2 and 3. Ashbey and Wangermann (1949), and Wangermann and Ashbey (1950) have reported similar cycles of senescence and rejuvenation in the vegetative growth of *Lemna minor* associated with periodic reduction/ increase in size and number of the frond cells.

Similar cycle of reduction (senescence) and increase (rejuvenation) in frond size at 1-2 month interval have observed in natural habitat by Pokharel (Bhattarai) and Jha (2017).

Maximum frond biomass per unit area was recorded in April (water temperature 26 °C) in water body 1, and in February (water temperature 27 °C) in water body 2 and 3 in the present study. Khondker *et al.* (1993 b) have reported that water temperature above 25 °C is detrimental to the growth of *S. polyrhiza*, whereas Pokharel (Bhattarai) and Jha (2016) have observed healthy growth of the test species even at 31.2 °C water temperature in a nutrient-poor roadside pool at Biratnagar, Nepal.

The shortest and the longest roots of *S. polyrhiza* occurred in May and March, respectively in water body 1, in December and February, respectively in water body 2, and in April and December, respectively in water body 3. Leng (1999) opined that roots either stabilize the frond on the water surface or assist the frond to obtain nutrients where these are in dilute concentrations. They tend to lengthen as mineral nutrients in water are exhausted.

4.2.2. GROWTH ON DIFFERENT WATER pH AND NUTRIENT MEDIA

In the present study, *S. polyrhiza* survived in the pH range of 4 - 7 and displayed optimum increase in fresh weight at pH 6 when grown alone, but had wider range of pH tolerance when grown with *Lemna aequinoctialis*. This finding is consistent with Mallik (2012) that mixed cultures can better tolerate potentially stressful changes in growth conditions in comparison to monocultures.

The best concentration of inorganic fertilizer per l of borewell water was N 10 mg, P 2 mg and K 2 mg for the growth of *S. polyrhiza* in the present study. Higher concentration of inorganic fertilizer had detrimental effect on frond growth. According to Thalek (2001), higher NPK concentration disturbs the plant water status which eventually results in osmotic stress and growth reduction.

Although appropriate concentration of glucose, sucrose, and xylose in water for the frond growth was 6 g/ l, 2 g/ l, and 6 g/ l, respectively, the best growth was observed in 2 g/ l sucrose concentration in the present study. Leng (1999) stated that like other

photosynthesizing organisms duckweeds grow with only requirements for minerals, utilizing solar energy to synthesize biomass ; however, they have the capacity to utilize pre-formed organic materials particularly sugars and can grow without sunlight when provided with such energy substrates. In practice the ability to use sugars in the medium as energy source is irrelevant, as in most aquatic systems sugars do not exist. However, they could be of some importance where industrial effluents need to be purified and duckweed is considered for this process (e.g., wastewater from sugar industry or wastewater from starch processing).

4.2.3. TURION FORMATION

Transfer of fronds from habitat (pH 7.1, N 4.30 mg/l, P 0.06 mg/ l, K 15.5 mg/ l) to borewell water (pH 6.4, N 0.20 mg/l, P < 0.05 mg/ l, K 3.26 mg/ l) under the decreasing day-length and temperature of December induced turion formation in *S. polyrhiza* in the present study. According to Appenroth (2002), for *S. polyrhiza* low phosphate levels and low temperatures both represent specific turion inducing factors having significant interactive effects, and these signals may replace the interactive effects of photoperiods and low temperature known from other hydrophytes in turion induction under natural conditions.

In general, the dormant phase of *S. polyrhiza* is represented by turions which circumvents sexual reproduction. There are conditions like temperature shifts due to seasons that can cause morphological change of fronds into turions, which are characterized by more starch, smaller vacuoles and air space (Landolt, E., 1986). Because the volume of intercellular air space shrinks and starch increases the density of the tissue, it can sink to the bottom of waters where the plant can survive even if top of the water freezes (Landolt, E., 1986). Turions can change back to fronds vegetatively using the starch as an energy source, demonstrating a highly evolved adaptation to the environment.

According to Wang and Messing (2012), turions of *S. polyrhiza* contain high starch content, small size of starch granules, and low lignin proportion, which provides a solid foundation for developing them as an alternative biofuel source.

4.2.4. FISHMEAL REPLACER

Currently, tilapia is the third largest group of farmed finfish species produced after the cyprinids and salmonids (Kingdon, J., 1989). It is a highly nutritional fish, rich in omega 3 oil which is good for heart ailments. The fish contains less bone than other common fish and has a good taste, making it popular among fish lovers. It can survive extreme conditions like low oxygen and abrupt changes in temperature (Kingdon 1989). However, as with the majority of finfish species produced within intensive farming systems the development of commercial aquafeeds for these species usually has been based upon the use of fishmeal as the main source of dietary protein which is too much costly for marginalized farmers. Besides, the demand for fishmeal in livestock and poultry feeds is ever-increasing.

Duckweed (*Lemna* spp.) has considerable potential for use as a fishmeal replacer within tilapia feeds (Mbagwu *et al.*, 1990) or as a complete diet in its live form (Edwards, 1987; Journey *et al.*, 1990; Wee, 1991). For example, Mbagwu *et al.*, (1990) reported that *Sarotherodon galilaeus* fed a diet (33 % protein) containing duckweed *Lemna paucicostata* as a partial protein source exhibited better growth and feed efficiency than fish fed a standard (40 % protein) diet. In the present study, tilapia (*Oreochromis niloticus*) reared solely on the conventional feed (mixture of wheat flour, soybean flour and fish meal in 2 : 2 : 1 ratio) had the highest increment in weight (65 %) in comparison to those reared on the mixture of conventional feed and duckweed (*S. polyrhiza*) powder in 1 : 1 ratio (37 %) or exclusively on duckweed powder (9 %). The poor growth performance of tilapia exclusively on duckweed meal might be attributed to very low concentration of fats (1.56 %) in comparison to the conventional feed (7.89 %). According to El Sayed and Tacon (1997), apart from being a good source of high quality protein, fishmeal is also a good source of essential fatty acids, digestible energy, macro and trace minerals, vitamins, and generally acts as a feeding stimulant for most finfish.

Even though duckweed meal as replacer of conventional feed (with fishmeal) reduced growth performance of tilapia, a mixture of duckweed powder and conventional feed in 1 : 1 ratio would be more economical on the basis of cost-benefit analysis (Pokharel (Bhattarai) and Jha, 2017).

4.2.5. WASTEWATER TREATMENT

Harvey and Fox (1973) reported removal of total nitrogen and total phosphorus from the secondary effluent by 86.5 and 67 %, respectively when *Lemna minor* was grown on the effluent over a 10 – day period. In the present study, however, culture of *S. polyrhiza* for 30 days removed only 25 % nitrogen and 22 % phosphorus from the wastewater. This lower value of nutrient removal might be attributed to low light intensity inside the laboratory that hampered optimum photosynthesis and nutrient assimilation by the duckweed (Pokharel (Bhattarai) and Jha, 2017).

In the present study, biomass of *S. polyrhiza* increased by 43.8 % after incubation on 2 mg/ l concentration of mercuric chloride for a week, whereas biomass increase was only 20.2 % in control in the same period. However, higher concentrations (> 2 mg/ l) of mercuric chloride had detrimental effect on the biomass. Similar findings have been reported for *Lemna minor* in the solutions of iron and copper by Jain and Jha (1989), and copper and nickel by Khellaf and Zerdauli (2009).

The chlorophyll a, chlorophyll b and carotenoid contents in *S. polyrhiza* incubated on 2 mg/ l mercuric chloride solution for a week were higher by 64, 29.6 and 59 %, respectively than control, whereas photosynthetic pigments decreased drastically at higher concentrations of mercuric chloride in the present study. The mechanism of effect of heavy metals on plant level of photosynthetic pigments may be owed to three reasons. First, heavy metals enter frond chloroplast (Sandilo *et al.*, 2001) and may get over - accumulated locally causing oxidative stress that will cause damages like peroxidation of chloroplast membranes (Puertas *et al.*, 2004). They can also directly destroy the structure and function of chloroplast by binding with - SH group of the enzymes and may also inhibit the overall chlorophyll biosynthesis by targeting Mg^{2+} and Fe^{2+} . Second, heavy metal ions inhibit uptake and transportation of other metal elements such as manganese and iron by antagonistic effects and therefore, the fronds lose their capacity to synthesize pigments (Das *et al.*, 1997). Third, heavy metals may activate pigment enzyme and accelerate the decomposition of pigment (Wenhua *et al.*, 2007).

As with many other aquatic plants, duckweeds grow best in water with high levels of nitrogen and phosphate. Eutrophication of a body of water can produce these

conditions and encourage the growth of duckweeds. As they grow, duckweeds absorb these nutrients from the water. Floating duckweeds are relatively easy to remove by skimming and this trait makes duckweeds useful in nutrient removal and bioremediation schemes.

4.2.6. DUCKWEED MANURE

In the present study, height, branch number, leaf number, leaf area, and dry weight of leaves and stems per plant were considerably higher in manure-treated than untreated individuals of both the test species. Fruit number per plant ranged between 17 (C) and 44 (7-D) in chilli, and 7 (C) and 15 (14-D) in brinjal plants although test species had no remarkable variation in fresh weight per fruit under different frequencies of manure treatment and control. The present findings are in agreement with Lot *et al.* (1979) that the duckweeds can be used as an organic fertilizer in agriculture via composting or by direct land application. Marschner (1986) opined that organic manures may increase soil fertility and thus the crop production potential possibly by changes in soil physico-chemical properties including structure, nutrient bioavailability, water holding capacity, cation exchange capacity, pH, microbial community and activity, etc.

Percentage contribution of roots to total dry weight per individual was more in control than the manure-treated chilli plants as roots had to penetrate deeper in soil in search of nutrients essential for the growth of aboveground parts. On the contrary, percentage contribution of roots to total dry weight in brinjal plants was least under 7-D manure treatment which might be attributed to active mineralization of the manure and release of nutrients for the plant growth.

The number of branches per plant was more in manure-treated than untreated individuals of both the test species, which probably was essential for proper arrangement of leaves in the canopy for increased photosynthetic efficiency (Raden *et al.*, 2009; Maghfoer *et al.*, 2014).

The leaf number and leaf area per plant of manure-treated individuals of both the test species increased considerably compared to those of the control samples. This came as

a result of the improved nutrition of the treated plants, which accelerated the growth and accumulation of biomass in the photosynthesizing organ.

Two main functions of organic manures in soils are the supply of nutrients and increase in the organic matter content of soils. The significance of organic manures as suppliers of nutrients to plant growth is determined by the rate of nutrient release; the higher the rate of nutrient release the less the soil organic matter (Subbiah *et al.*, 1985). In the present study soil organic matter was recorded more in manure-treated than control chilli pots, but less than control in 14-D and 21-D manure-treated brinjal pots. It was probably due to faster mineralization of manure under brinjal than chilli cultivation.

Ullah *et al.* (2008) reported decrease in soil pH with organic manure application, and increase with chemical fertilizer application. The findings of the present study were in agreement with above report in the case of chilli, but under brinjal cultivation soil pH was slightly more than control under 7-D and 14-D manurings. It might be due to induced variations in soil organic matter pool by the brinjal plants to meet their nutrient demands.

Nitrogen is essential for plant growth as it is a constituent of all proteins and nucleic acids and hence of all protoplasm. It is generally taken up by plants either as ammonium or as nitrate ions, but the absorbed nitrate is rapidly reduced to ammonium through a molybdenum-containing enzyme. The ammonium ions and some of the carbohydrates synthesized in the leaves are converted into amino acids, mainly in the green leaf itself. Hence, as the level of N supply increases compared with other nutrients, the extra protein produced allows the plant leaves to grow larger surface area available for photosynthesis, and in fact, over a considerable range of N supply for many crops, the amount of leaf area available for photosynthesis is roughly proportional to the amount of N supplied (Russell, 1973). In the present study, total N in soil samples was considerably higher in manured pots (7-D and 14-D) planted with chilli, but except 7-D, soil N concentration was higher in control than 14-D and 21-D manure-treated pots under brinjal cultivation indicating greater demand of this nutrient by brinjal plants.

Phosphorus, as ortho-phosphate (H_2PO_4^-), plays a fundamental role in a large number of enzymic reactions that depend on phosphorylation. Possibly for this reason it is a constituent of the cell nucleus and is essential for cell division and for the development of meristem tissue. Phosphate seems to increase leaf area without affecting the power of the leaves to transport carbohydrates to the roots, and it thus differs from nitrogen manuring, which also increases leaf growth but reduces their power of sending carbohydrates to roots (Russell 1973). In the present study, phosphorus concentration increased tremendously in manure-treated pots planted with the test species. It might be attributed to the P content in the manure, and relative inefficiency of the test species in using this nutrient. In general, if a phosphate fertilizer is added to a soil, an annual crop usually takes up only about 5 to 10 per cent of the phosphate added, even if it responds well to the phosphate, though phosphate-poor soils may give higher recoveries (Marschner, 1986).

Potassium is a vitally important mineral element which the plant requires in the largest quantity (Obigbesan, 1977). It differs from N and P, however, in not being a constituent of the plant fabric. It is important in the synthesis of amino acids and proteins from ammonium ions, and an adequate supply of K in the leaf is essential for the photosynthetic process to go on efficiently. In the present study, K concentration was lower in manure-treated soils than the control probably due to its removal in large quantity by the test species. Although it appears that duckweed manure in combination with chemical K fertilizer can meet optimum K demand of the crops, excess K in the soil due to too high a level of K manuring will reduce the amount of other cations the crop can take up, and this may lead to crop growth being badly upset by the induced deficiency of other cations.

In brief, biomass and nutrient concentration in *S. polyrhiza* depended on nutrient concentration of habitat water, the fronds displayed cycles of senescence and rejuvenation and formed turions when incubated on nutrient deficient water under low temperature. The duckweed had a wider range of pH tolerance when grown with *L. aequinoctialis* than alone, and had optimum growth on N : P : K fertilizer in 5 : 1 : 1 ratio (N 10 mg, P 2 mg, K 2 mg per litre of borewell water) as well as on 2 g sucrose per litre borewell water. The duckweed partially removed N, P, K from wastewater contaminated with rotting vegetables, and displayed bioremediation capability up to 2

mg/l concentration of mercuric chloride. The duckweed meal partially replaced fishmeal (the most costly ingredient in conventional fish feed); and duckweed manure besides promoting growth and fruit yields of brinjal and chilli crops, increased phosphorus concentration in soil substantially.

CHAPTER 5
CONCLUSIONS AND
RECOMMENDATIONS

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

Distinct cycles of senescence and rejuvenation were observed in *in-situ* growth of the *Spirodela polyrhiza* colonies in the present study.

The major function of *S. polyrhiza* roots was to stabilize the frond on the water surface as increase/ decrease in root length had no correlation with decrease/ increase in nutrient concentration in water

Comparatively, the highest fresh biomass (823 g/m²) and nutrient concentration (protein 19.6 %, phosphorus 0.9 %, and potassium 2.1 %) in *Spirodela* occurred in winter (January) when concentrations of nitrogen, phosphorus and potassium were also highest (in comparison to summer and rainy seasons) in habitat water.

Spirodela tolerated the pH range of water only between 4 - 7 when incubated alone, and optimum growth occurred at pH 6. However, when incubated in mixture with *Lemna aequinoctialis*, *S. polyrhiza* survived in 2 - 10 range of water pH although gain in fresh weight at pH 6 was comparatively less than in pure culture after one month of incubation.

The best concentration of inorganic fertilizer per l of borewell water was N 10 mg, P 2 mg and K 2 mg for the growth of *Spirodela*. Higher concentrations of inorganic fertilizer had detrimental effect on frond growth.

In comparison to different concentrations of glucose, sucrose and xylose in borewell water, the best growth of *S. polyrhiza* was observed on 2 g/l sucrose concentration in the present study, indicating suitability of *S. polyrhiza* as purifier of wastewater from sugar industry or wastewater from starch processing.

After 3 week incubation on low-nutrient borewell water in December (decreasing day-length and temperature), the normal fronds of *S. polyrhiza* produced turions. Basically the turions are the overwintering buds that develop into normal fronds on arrival of the favourable season; however, due to high starch content, small size of starch granules,

and low lignin proportion, these turions can be used as an alternative biofuel source also.

In the present study, tilapia (*Oreochromis niloticus*) reared solely on the conventional feed (mixture of wheat flour, soybean flour and fishmeal in 2 : 2 : 1 ratio) had the highest increment in weight than those reared on the mixture of conventional feed and duckweed (*Spirodela*) powder in 1 : 1 ratio or exclusively on duckweed meal. However, a mixture of duckweed meal and conventional feed in 1 : 1 ratio may be the more economical feed for tilapia farming on the basis of cost-benefit analysis.

The fresh weight of *Spirodela* fronds increased from initial 1 g to 3.5 g after incubation on wastewater for 30 days in shade, whereas concentrations of nitrogen, phosphorus and potassium in the wastewater decreased by 25, 24 and 22 %, respectively.

In the present study, both biomass and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) in *S. polyrhiza* increased significantly after a week incubation on 2 mg/l concentration of mercuric chloride in borewell water. It indicated the suitability of *S. polyrhiza* for treatment of wastewaters with dilute concentrations of heavy metals.

In the present study, the height, branch number, leaf number, leaf area, and leaf weight (dry) per plant were significantly highest in both brinjal and chilli crops treated with duckweed (mixture of *S. polyrhiza* and *Lemna aequinoctialis*) manure at 7-days intervals. The best frequency of manuring for fruit production was 7-day intervals for brinjal plants. The soils under manure treatments accumulated astoundingly high concentration of phosphorus.

5.2. RECOMMENDATIONS

The growing awareness of water pollution and its threat to the ecology of a region and agriculture *per se* has also focused attention on potential biological mechanisms for cleansing water of these impurities making it potable and available for reuse. There are thousands of derelict ponds polluted to eutrophication levels in Nepal alone that could potentially be cleansed of much of their pollutants and resurrected for duckweed aquaculture and fish farming. To resurrect derelict ponds, the approach might be to first establish duckweed aquaculture as a source of nutrient-rich animal feed or fertilizer for crop production, and, as the pond's oxygen levels rise with harvesting of the duckweed, to induce fish farming either in part of the pond or in adjacent (clean water) ponds.

Duckweeds will remain an unutilized resource unless the farmers are familiar with their economic and environmental values. There is a vast need for popularization, market, and research support for duckweeds.

CHAPTER 6
SUMMARY

6. SUMMARY

The growth patterns (in-situ and ex-situ), conditions for turion (overwintering buds) formation, use as fishmeal replacer in tilapia (*Oriochromis niloticus*) farming, water cleansing efficiency, response to mercury (a heavy metal) stress, and use as manure (in brinjal and chilli cultivation) of *Spirodela polyrhiza* – the most ubiquitous but neglected duckweed of eutrophic water bodies at Biratnagar, Nepal were investigated in the present study.

The duckweed displayed distinct cycle of senescence and rejuvenation and had the highest biomass and nutrients (protein, phosphorus and potassium) in winter (January) when the concentration of dissolved nitrogen phosphorus and potassium in the habitat water were also the highest in comparison to other season.

For the growth of the duckweed, the best water pH was 6; the best concentration of inorganic fertilizer per litre of borewell water was N 10 mg, P 2 mg and K 2 mg; and of the various concentration of glucose, sucrose and xylose in borewell water, the best growth was observed in 2 g/l sucrose concentration.

Three weeks after transfer from nutrients-rich habitat water and subsequent incubation on nutrient poor borewell water in December, the normal duckweed fronds produced turions.

The fresh weight gain of tilapia fingerlings reared on three different feeds (conventional feed: a mixture of wheat flour, soybean flour and powdered local fishes in 2: 2: 1 ratio; powdered greater duckweed meal; and 50 % conventional feed plus 50 % duckweed meal) for 21 days was although the highest on conventional feed, nevertheless the mixture of duckweed meal and conventional feed in 1:1 ratio appeared to reduce the farming cost of tilapia.

The duckweed considerably reduce the concentration of N, P and K in water contaminated with organic wastes; and appear as a reliable species for the treatment of wastewater with dilute concentration (up to 2 mg/l) of heavy metals.

The number of leaves, leaf area, number of branches, fruit yield and total dry weight per plant of both brinjal and chilli crops treated with semi-liquid duckweed manure at 7-day and 14-day intervals were much more than control. Similarly phosphorus concentration in soil under different frequencies of duckweed manuring increased astoundingly.

REFERENCES

REFERENCES

- Ali, Z., and Ahmad, M. (2016). Duckweed: An efficient hyper accumulator of heavy metals in water bodies. In P. Ahmad (ed.) *Plant Metal Interaction: Emerging Remediation Techniques*. Elsevier Inc., Amsterdam. pp. 411-429.
- APHA (American Public Health Association). (1990). *Standard Methods for the examination of water and waste water*, 16th Edition. APHA, Inc., New Work, USA.
- Appenroth, K. – J., Hertel, W., Jungnicke F., and Augsten, H. (1989). Influence of nutrient deficiency and light on turion formation in *Spirodela polyrhiza* (L.) Schleiden. *Biochemie und Physiologie der Pflanzen*, **184**: 395-403.
- Appenroth, K. – J., Teller, S. and Horn, M. (1996). Photophysiology of turion formation and germination in *Spirodela polyrhiza*. *Biologia Plantarum*, **38**(1): 95-106.
- Appenroth, K. -J. (2002). Co-action of temperature and phosphate in inducing turion formation in *Spirodela polyrhiza*(greater duckweed). *Plant, Cell & Environment*, **25**(9): 1079-1085.
- Appenroth, K. – J., and Nickel, G. (2009). Turion formation in *Spirodela polyrhiza*. The environmental signals that induce the development process in nature. *Physiologia Plantarum*, **138**(3): 312-320.
- Appenroth, K. – J., Sree, K. S., Bohm, V., Hammann, S., Vetter, W., Leiterer, M., and Jehreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chemistry*, **217**: 266-273.
- Ashbey, E., and Wangermann, E. (1949). Senescence and rejuvenation in *Lemna minor*. *Nature*, **164**: 187
- BFRI. 1997. *Research Progress Report: January – August, 1997*. Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh.
- Constance, D. H. (2010). Sustainable agriculture in the United State: A critical examination of a conested process. *Sustainability*, **2**: 48-72.

- Cook, C. D. K. (1996). *Aquatic and Wetland Plants of India*. Oxford University Press, New Delhi.
- Culley, D. D., and Epps, A. E. (1973). Use of duckweeds for waste treatment and animal feed. *Journal of the Water Pollution Control Federation*, **45**: 337-347.
- Culley, D. D., Rejmenkova, E., Kvet, J., and Frye, J. B. (1981). Production, chemical quality and use of duckweeds (*Lemnaceae*) in aquaculture, waste management, and animal feeds. *Journal of the World Mariculture Society*, **12**(2): 27-49.
- Culley, D. D., Gholson, J. H., Chisholm, T. S., Standifer, L. C., and Epps, E. A. (1978). *Water Quality Renovation of Animal Waste Lagoons Utilizing Aquatic Plants*. US Environmental protection Agency, Oklahoma.
- Das, P., Samantaray, S., and Rout, G. R. (1997). Studies on cadmium toxicity in plants : a review. *Environmental Pollution*, **98**: 29-36.
- Davis, K. M., Drake, K. D., and Maier, K. J. (2002). Toxicity of boron to the duckweed *Spirodela polyrhiza*. *Chemosphere*, **48**: 615-620.
- DeBusk, T. A., and Reddy, K. R. (1987). BOD removal in floating aquatic macrophyte-based wastewater treatment systems. *Water Science and Technology*, **19**: 273-279.
- DWRP. (1998). *Duckweed in Bangladesh*. Duckweed Research Project, Ministry of Fisheries and Livestock and Royal Netherlands Embassy, Dhaka.
- Edwards, P. (1987). Use of terrestrial vegetation and aquatic macrophytes in aquaculture. In D. J. W. Moriarty and R. S. V. Pullin (eds.) *Detritus and Microbial Ecology in Aquaculture*. ICLARM Conference Proceedings No. 14, Manila. pp. 311-335.
- Edwards, P., Hassan, M. S., Chao, C. H., and Pacharaprakiti, C. (1992). Cultivation of duckweeds in septage – loaded earthen ponds. *Bioresource Technology*, **40**: 109-117.

- El Sayed, A. F. M., and Tacon A. G. J. (1997). Fishmeal replacers for tilapia : a review. In A. G. J. Tacon and B. Basuro (eds.). *Feeding Tomorrow's Fish*. CIHEAM, Zaragoza. pp. 205-224.
- Fasakin, E. A., Balogun, A. M., and Fasure, B. E. (1999). Use of duckweed, *Spirodela polyrhiza* (L.) Schleiden, as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* L. *Aquaculture Research*, **30**: 313-318.
- Franceschi, V. R. (1989). Calcium oxalate formation is a rapid and reversible process in *Lemna minor* L. *Protoplasma*, **148**: 130-137.
- Furman, N. H. (ed.). (1962). *Standard Method of Chemical Analysis*, 6th Edition. New Jersey: D. Van Nostrand Co.
- Gaigher, I. G., and Short, R. (1986). An evaluation of duckweed (Lemnaceae) as a candidate for aquaculture in South Africa. In R. D. Wamsley and J. G. Wan (eds.) *Aquaculture 1980*. Report Series no 15, CSIRO, Canberra. pp. 81-90.
- Galkina Abdullaev, D. A., and Zacharova, V. L. (1965). Biological and feed features of duckweeds. *Uzbekistan Biological Journal*, **3**: 44-47.
- Gijzen, H. J. and Khondker, M. (1997). An overview of the ecology, physiology, cultivation and applications of duckweed. *Inception Report. Annex 1. Literature Review*. Duckweed Research Project, Dhaka.
- Gopal, B., and Chamanlal. (1991). Distribution of aquatic macrophytes in polluted water bodies and their bio-indicator value. *Verhandlungen des Internationalen Vereins fur Limnologie*, **24**: 2125-2129.
- GON (Government of Nepal). (2019). *Normals from 1989-2018*. Kathmand: Department of Hydrology and Meteorology.
- Hara, H., W. T. Stearn and L. H. J. Williams (eds.). 1978. *An Enumeration of the Flowering Plants of Nepal*, Vol. 1. British Museum (Natural History), London.
- Harvey, R. M., and Fox, J. L. (1973). Nutrient removal using *Lemna minor*. *Journal of Water Pollution Control Federation*, **45**(9): 1928-1938.

- Harvey, R. M., and Fox, J. L. (1973). Nutrient removal using *Lemna minor*. *Journal of Water Pollution Control Federation*, **45**(9): 1928-1938.
- Hasan, M. R., and Chakrabarti, R. (2009). *Use of Algae and Aquatic Macrophytes as Feed in Small-scale Aquaculture: A Review*. Food and Agriculture Organization of the United Nations, Rome.
- Hassan, M. S., and Edwards, P. (1992). Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) as feed for Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **104**: 315-326.
- Henssen, A. (1954). Die dauerorgane von *Spirodela polyrrhiza* (L.) Schleid. in physiologischer Betrachtung. *Flora*, **141**: 523-566.
- Hepher, B., and Pruginin, Y. (1979). *Guide to Fish Culture in Israel: 4. Fertilization, Manuring and Feeding*. Foreign Training Department, Jerusalem, Israel.
- Hillman, W. S., and Culley, D. D. (1978). The uses of duckweed. *American Scientist*, **66**: 442-451.
- Hutchinson, G. E. (1975). *A Treatise on Limnology, Vol. 3: Limnological Botany*. New York: John Wiley & Sons.
- Iqbal, S. (1999). *Duckweed Aquaculture: Potentials, Possibilities and Limitations for Combined Wastewater Treatment and Animal Feed Production in Developing Countries*. SANDEC Report No. 6/99. Department of Water & Sanitation in Developing Countries, Swiss Federal Institute for Environmental Science & Technology, Duebendorf.
- Islam, A. K. M. N., and Paul, S. N. (1977). Limnological studies on *Wolffia arrhiza* (L.) Wimm. *Journal of the Asiatic Society Bangaldesh (Sciences)*, **3**: 111-123.
- Islam, A.K.M.N., and Khondker, M. (1991). Preliminary limonological investigations of some polluted waters covered by duckweeds. *Bangladesh Journal of Botany*, **20**: 73-75.

- Jacobs, D. L. (1947). An ecological life history of *Spirodela polyrhiza* (greater duckweed) with emphasis on the turion phase. *Ecological Monographs*, **17**(4): 437-469.
- Jain, S. K., and Jha, N. K. (1989). Removal of some heavy metals from polluted water by aquatic plants: Studies on duckweed and water velvet. *Biological Wastes*, **28** (2): 115-126.
- Jha, S. (1990). *Conservation for Development in Nepal*. National Book Organisation, New Delhi.
- Jha, S. (2010). Seasonal Changes in physico-chemical properties of soil and forage nutrients in a lowland Nepalese grassland. *Pakistan Journal of Plant Science*, **16**(2): 51-63.
- Jha, S., and Niraula, B. (1998). Effect of various industrial effluents and municipal sewage on germination and growth of rice and blackgram. *Tribhuvan University Journal*, **21**(1): 63-60.
- Jha, S., and Pokharel (Bhattarai), B. (2015). Duckweed : the hidden treasure. *Nepalese Journal of Biosciences*, **5**(1): 1-24.
- Jha, S., and Jha, P. K. (2000). Contribution to the flora of Morang district and adjoining areas of Nepal. *Lidia*, (A Norwegian Journal of Botany) **5**(1-2): 25-64.
- Journey, W. K., Skillicorn., and Spira, W. (1990). *Duckweed Aquaculture : A New Aquatic Farming System for Developing Countries*. Washington D.C.: The World Bank.
- Kabir, A. N. M. A., Hossain, M. A., and Rahman, M. S. (2009). Use of duckweed as feed for fishes in polyculture. *Journal of Agriculture and Rural Development*, **7**(1-2): 157-160.
- Kaul, V., and Bakaya, U. (1973). The noxious, floating, lemnid *Salvinia* aquatic weed complex in Kashmir. In C. R. Varshney and J. Rzoska (eds.) *Aquatic weeds in S. E. Asia*. The Hague, Junk.

- Khellaf, N., and Zerdauli, M. (2009). Growth response of the duckweed *Lemna minor* to heavy metal pollution. *Iranian Journal of Environment, Health, Science & Engineering*, **6**(3): 161-166.
- Khondker, M., Islam, A. K. M. N., and Nahar, N. (1993 a). Study on the biomass of *Spirodella polyrhiza* and the related limnological factors of some polluted waters. In M. S. Khan, M. A. Aziz Khan, S. Hadiuzzaman and A. Aziz (eds.) *Plants for the Environment. Proceedings of the 7th Botanical Conference*, 13 - 14 December 1992. Bangladesh Botanical Society, Dhaka. pp. 37-40.
- Khondker, M., Islam, A. K. M. N., and Nahar, N. (1993 b). A preliminary study on the growth rate of *Spirodela polyrhiza*. *Dhaka University Journal of Biological Sciences*, **2**: 197-200.
- Khondker, M., Islam, A. K. M. N., and Makhnun, A. D. (1994). *Lemna perpusilla* screening on habitat limnology. *Bangladesh Journal of Botany*, **23**: 91-106.
- Kingdon, J. (1989). *Island Africa : The Evolution of Africa's Rare Plants and Animals*. New Jersey: Princeton University Press.
- Koirala, U. (2015). *Ecological Study of Wetland Macrophytes Occurring in Morang District of Nepal*. Ph. D. Thesis, Tribhuvan University, Kathmandu.
- Korner, S., and Vermaat, J. E. (1998). The relative importance of *Lemna gibba* L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Water Research*, **32**(12): 3651-3661.
- Kuhdorf, K., Jetschke, G., Ballani, L., and Appenroth, K. – J. (2014). The clonal dependence of turion formation in the duckweed *Spirodela polyrhiza*- an ecogeographical approach. *Physiologia Plantarum*, **150**: 46-54.
- Landolt, E. (1986). *The family of Lemnaceae - A Monographic Study: Morphology, Karyology, Ecology, Geographic distribution, Systematic position, Nomenclature, Descriptions*, Vol. 2. Veröffentlichungen des Geobotanisches Institute der Eidgenössische Technische Hochschule, Stiftung Ruebel, Zurich.

- Landolt, E. (2006). Duckweed. In *Flora of North America North of Mexico*, Vol. 22. Flora of North America Editorial Committee, New York and Oxford.
- Landolt, E. and Kandeler, R. (1987). *The family of Lemnaceae - A Monographic Study: Phytochemistry, Physiology, Application and Bibliography*, Vol. 4. Veroffentlichungen des Geobotanisches Institut der Eidgenossische Technische Hochschule, Stiftung Ruebel, Zurich.
- Leng, R. A., Stambolie, J. H., and Bell, R. (1994). Duckweed - a potential high-protein feed resource for domestic animals and fish. In *Proceedings of the 7th Animal Science Congress of the Asian - Australasian Association of Animal Production Societies (AAAP) Conference*. Indonesian Society of Animal Science, Jakarta. pp. 103-114.
- Leng, R. A. (1999). *Duckweed: A Tiny Aquatic Plant with Enormous Potential for Agriculture and Environment*. Roam: Food and Agriculture Organization of the United Nations.
- Lin, Y., Zhang, M., Tian, Y., Zhao, M., Zhang, B., M., Zeng, K., and Yin, B. (1917). Duckweed (*Spirodela polyrhiza*) as green manure for increasing yield and reducing nitrogen loss in rice production. *Field Crops Research*, **214**: 273-282.
- Lot, A., Novelo, A., and Quiroz, A. (1979). The chinampa: an agricultural system that utilizes aquatic plants. *Journal of Aquatic Plant Management*, **17**: 74-75.
- Luond, A. (1980). Effects of nitrogen and phosphorus upon the growth of some *Lemnaceae*. In E. Landolt (ed.) *Biosystematic Investigations in the Family of Duckweeds (Lemnaceae)*. Veroffentlichungen des Geobotanisches Institute der Eidgenossische Technische Hochschule, Stiftung Ruebel, Zurich. pp. 118-141.
- Maghoer, M. D., Soelistyono, R., and Herlina, N. (2014). Growth and yield of eggplant (*Solanum melongena L.*) on various combinations of N - source and number of main branch. *Agrivita*, **36**(3): 285-294.
- Mahofa, R., Kapenzi, A., and Masaka, J. (2014). The effects of different types of duckweed manure on height and yield of Floradade tomatoes. *Midlands State University Journal of Science, Agriculture and Technology*, **5**(1): 135-152.

- Majid, F. Z., Khatun, R., Akhtar, N., and Rahman, A. S. M. A. (1992). Aquatic weeds as a source of protein in Bangladesh. *Bangladesh Journal of Scientific and Industrial Research*, **27**: 103-111.
- Malek, L., and Cossins, E. (1983). Senescence, turion development, and turion germination in nitrate and sulphate - deficient *Spirodela polyrhiza*. Relationship between nutrient availability and exogenous cytokinins. *Canadian Journal of Botany*, **61**: 1887-1897.
- Mallik, A. U. (2012). *Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystem*. Birkhauser Verlag, Switzerland.
- Marschner, H. (1986). *Mineral Nutrition of Higher Plants*. New York: Academic Press.
- Mbagwu, I. G., Okoye, F. C., and Adinji, H. A. (1990). *Studies on the use of Duckweed (Lemna paucicostata Helgam.) as Fish Food*. Technical Report 22, Freshwater Fisheries Research, Bussa, Nigeria.
- Mohedano, R. A., and Filho, P. B. (2012). High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology*, **112**: 98-104.
- Nassar, H. F., Shaban, A. M., Bassem, S. M., and Abdel-Gawad, F. K. (2015). Utilisation of duckweed in nutrient removal from agricultural wastewater and producing alternative economic animal fodder. *Der Pharma Chemica*, **7**(12): 280-285.
- Naumann, B., and Appenroth, K. -J. (2007). Growth rate based dose-response relationships and EC-values of ten heavy metals using the duckweed growth inhibition test (ISO 20079) with *Lemna minor* L. clone St. *Journal of Plant Physiology*, **164**(12): 1656-1664.
- Newton, R. J., Shelton, D. R., Disharoon, S., and Duffey, J. E. (1978). Turion formation and germination in *Spirodela polyrhiza*. *American Journal of Botany*, **65**: 421-428.

- Obigbeson, G. O. (1977). Investigations on Nigerian root and tuber crops. Responses of fertilizer in western Nigeria. *Journal of Agriculture Science*, **89**: 23-27.
- Olah, V., Hepp, A., Lakatos, G., and Meszaros, I. (2014). Cadmium – induced turion formation of *Spirodela polyrhiza* (L.) Schleiden. *Acta Biologia Szegediensis*, **58**(2): 103-108.
- Peach, K., and Tracey M. V. (1956). *Modern Methods of Plant Analysis*, Vol. 1. Berlin: Springer – Verlag.
- Perry, T. O. (1968). Dormancy, turion formation, and germination by different clones of *Spirodela polyrhiza*. *Plant Physiology*, **43**: 1866-1869.
- Perry, T. O., and Byrne, O. R. (1969). Turion induction in *Spirodela polyrhiza* by abscisic acid. *Plant Physiology*, **44**: 784-785.
- Piper, C. S. (1944). *Soil and Plant Analysis*. The University of Adelaide, Adelaide, Australia.
- Pokharel (Bhattarai), B., and Jha, S. (2016). Seasonal variations in biomass and nutritive value of two duckweed species at Biratnagar, Nepal. *Geobios*, **43**: 4-7.
- Pokharel (Bhattarai), B., & Jha, S. (2017). Effect of duckweed manure on growth and yield of chilli and brinjal plants. *Geobios*, **44**: 31-34.
- Pokharel (Bhattarai), B., & Jha, S. (2017). Growth on different media and water cleansing efficiency of *Spirodela polyrhiza* (L.) Schleiden. *Geobios*, **44**: 142-148.
- Pokharel (Bhattarai) B., Niroula, B., and Jha, S. (2017). Use of greater duckweed as fish feed for Tilapia fish. *Nepalese Journal of Biosciences*, **7**: 117-119.
- Porath, D., and Koton, A. (1977). Enhancement of protein production in fish ponds with duckweed (Lemnaceae). *Israeli Journal of Botany*, **26**: 51.
- Puertas, R. M. C., Rodriguez, S. M., and Corpas, F. J. (2004). Cadmium - induced subcellular accumulation of oxygen and hydrogen peroxide in pea leaves. *Plant, Cell & Environment*, **27**: 1122-1134.

- Raden, I., Purwoko, B. S., Hariadi, M. G., and Santosa, E. (2009). Effect of pruning high and number of primary branches maintained on jatropha (*Jatropha curcas L.*) oil production. *Journal of Agronomy (Indonesia)*, **37**(2): 159-166.
- Reddy, K. R., and DeBusk, W. F. (1985). Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: 1. Water hyacinth, water lettuce, and pennywort. *Economic Botany*, **38**: 229-239.
- Reddy, K. R., and DeBusk, W. F. (1985). *Textbook of Fish Genetics and Biotechnology*. New Delhi: Indian Council of Agricultural Research.
- Rejmankova, E. (1975). Comparison of *Lemna gibba* and *Lemna minor* from the production viewpoint. *Aquatic Botany*, **1**: 423-427.
- Robinette, H. R., Brunson, M. W., and Day, E. J. (1980). Use of duckweed in diets of channel catfish. In *Proceeding of the 13th Annual Conference of the Southeastern Association of Fish and Wildlife Agencies (SEAFWA)*. pp. 108-114.
- Russell, E. W. (1973). *Soil Conditions and Plant Growth*, 10th Edition. Longman Group Limited, London, UK.
- Said, M. Z., Culley, D. D., Standifer, L. C., Epps, E. A. Myers, R.W., and Bonney, S. A. (1979). Effect of harvest rate, waste loading and stocking density on the yield of duckweeds. *Proceedings of the World Mariculture Society*, **10**: 769-780.
- Sandalio, L. M., Dalurzo, H. C., and Gomez, M. (2001). Cadmium - induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, **52**: 2115-2126.
- Sekomo, C. B., and Lens, P. N. L. (2014). Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. *Ecological Engineering*, **44**: 102-110.
- Sheh - May, T., Boyce, P. C., Upson, T. M., Barabe, D., Bruneau, A., Forest, F., and Parker, J. S. (2004). Intergeneric and intrafamilial phylogeny of subfamily

- Monosteroideae (Araceae) revealed by chloroplast sequences. *American Journal of Botany*, **91**: 490-498.
- Shireman, J. V., Colle, D. E., and Rottmann, R. W. (1977). Intensive culture of grass carp, *Ctenopharyngodon idella* in circular tanks. *Journal of Fish Biology*, **11**: 267-272.
- Sibasaki, T., and Oda, Y. (1979). Heterogeneity of dormancy in the turions of *Spirodela polyrhiza*. *Plant & Cell Physiology*, **20**: 563-571.
- Siwakoti, M., and Verma, S. K. (1999). *Plant Diversity of Eastern Nepal/Flora of Plains of Eastern Nepal*. Dehra Dun: Bishen Singh Mahendra Pal Singh.
- Skillicorn, P., Spira, W., & Journey, W. (1993). *Duckweed Aquaculture: A New Aquatic Farming System for Developing Countries*. Washington, D.C: The World Bank.
- Smart, C. C., Fleming, A. J., Chaloupkova, K., and Hanke, D. E. (1995). The physiological role of abscisic acid in eliciting turion morphogenesis. *Plant Physiology*, **108**: 623-632.
- Smart, C. C., and Trewavas, A. J. (2006). Abscisic acid induced turion formation in *Spirodela polyrhiza* L. I. Production and development of the turion. *Plant, Cell and Environment*, **6**(6): 507-514.
- Steward, G. R. (1969). Abscisic acid and morphogenesis in *Lemna polyrhiza* L. *Nature* **221**: 61-62.
- Stephenson, M., Turner, G., Pope, P., Kolt, J., Knight, A., and Tchobanoglous, G. (1980). *The Use and Potential of Aquatic Species For Wastewater Treatment: The Environmental Requirements of Aquatic Plants*. California State Water Resources Control Board, Sacramento.
- Subbiah, K., Sundararajan, S., Muthuswami, S., and Perumal, R. (1985). Responses of tomato and brinjal to varying levels of FYM and macronutrients under different fertility status of soil. *South Indian Horticulture* **33**(3): 198-205.

- Sutton, D. L., and Ornes, W. H. (1975). Phosphorus removal from static sewage effluent using duckweed. *Journal of Environmental Quality*, **4**: 367-370.
- Thalek, M., Mlinarec, J., Vidakovic – Cifrek, Z., & Zelencic, B. (2001). The effect of salinity and osmotic stress on duckweed *Lemna minor*. L. *Acta Botanica Croatica*, **60**(2): 237-244.
- Ullah, M. S., Islam, M. S., Islam, M. A., and Haque, T. (2008). Effects of organic manures and chemical fertilizers on the yield of brinjal and soil properties. *Journal of Bangladesh Agricultural University*, **6**(2): 271-276.
- Upadhyay, B. P., Jha, S., Koirala, U., and Niroula, B. (2011). Inventory of ten aquatic habitats of Biratnagar township, Nepal. *Geobios* **38**(4): 283-288.
- US Geological Survey. (1974). *Methods for collection and Analysis of Water samples for Dissolved Minerals and Gases*. Washington D.C.: US Geological survey.
- Van der Does, J., and Klink, F. J. (1991). Excessive growth of *Lemnaceae* and *Azolla* in ditches observed by false colour teledetection. *Verhandlungen des Internationalen Vereins fur Limnologie*, **24**: 2683-2688.
- Velten, S., Leventon, J., Jager, N., & Newig, J. (2015). What is sustainable agriculture? A systematic review. *Sustainability*, **7**: 7833-7865.
- Verma, R., and Suthar, S. (2015). Lead and cadmium removal from water using duckweed - *Lemna minor* L.: Impact of pH and initial metal load. *Alexandria Engineering Journal*, **54**(4): 1297-1304.
- Walkley, A. J., & Black, I. A. (1934). An examination of method for determining organic carbon and nitrogen in soil. *Journal of Agricultural Science*, **25**, 589-609.
- Wangermann, E., and Ashbey, E. (1950). Morphogenesis in *Lemna minor*. *Proceedings Linnean Society of London*, **162**: 10-13.
- Wang, W., and Messing, J. (2012). Analysis of ADP-glucose pyrophosphorylase expression during turion formation induced by abscisic acid in *Spirodela polyrhiza* (greater duckweed). *BMC Plant Biology*, **12**: 274-290.

- Wee, K. L. (1991). Use of non-conventional feedstuff of plant origin as fish feeds : is it practical and economically feasible? In S. S. De Silva (ed.) *Fish Nutrition Research in Asia*. Asian Fisheries Society, Manila, Philippines. pp. 13-32.
- Wenhua, H., Xiao, C., Guanling, G., Quinhui, W., and Chein, C. C. (2007). Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). *Plant Physiology and Biochemistry*, **45**: 62-69.
- Willard, H. H., Merritt, L. L., and Dean, J. A. (1974) *Instrumental Method of Analysis*, 5th Edition. New Jersey: D. Van Nostrand Co.
- Wolff, P., and Landolt, E. (1994). Spread of *Lemna turionifera* (Lemnaceae), the red duckweed in Poland. *Fragmenta Floristica et Geobotanica*, **39**: 439-451.
- Zaher, M., Begum, N. N., Hoq, M. E., and Bhuiyan, A. K. A. (1995). Suitability of duckweed, *Lemna minor* as an ingredient in the feed of tilapia, *Oreochromis niloticus*. *Bangladesh Journal of Zoology*, **23**: 7-12.
- Zhao, Z., and Zhao, Y. (2014). The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. *Bioresource Technology* **167**: 383-389.
- Zirschky, J., and Reed, S. C. (1988). The use of duckweed for wastewater treatment. *Journal of the Water Pollution Control Federation*, **60**: 1254-1258.
- Zobel, D. B., Jha, P. K., Yadav, U. K., and Behan, M. J. (1987). *A Practical Manual for Ecology* ., Kathmandu: Ratna Book Distributors.
- Zutshi, D. P., and Vass, K. K. (1973). Ecology of macrophytic vegetation of Kashmir lake. In C. R. Varshney and J. Rzoska (eds). *Aquatic weeds in S. E. Asia*. Junk, The Hague. Pp. 141-146.

APPENDIX 1

SCIENTIFIC PUBLICATION

(PUBLISHED PAPERS BASED ON

THE PRESENT STUDY)

APPENDIX 1: PUBLISHED PAPERS BASED ON THE PRESENT STUDY

- 1.1 S. Jha. and B. Pokharel (Bhattarai). 2015. Duckweed: the hidden treasure. *Nepalese Journal of Biosciences* **5** (1): 1 - 24.
- 1.2 B. Pokharel (Bhattarai) and S. Jha. 2016. Seasonal variations in biomass and nutritive value of two duckweed species at Biratnagar, Nepal. *Geobios* **43** : 4 - 7.
- 1.3 B. Pokharel (Bhattarai) and S. Jha. 2017. Growth on different media and water cleansing efficiency of *Spirodela polyrhiza* (L.) Schleiden. *Geobios* **44** (3 - 4) : 142 -148.
- 1.4 B. Pokharel (Bhattarai) and S. Jha. 2017. Effect of duckweed manure on growth and yield of chilli and brinjal plants. *Geobios* **44** (1 - 2): 31 - 34.
- 1.5 B. Pokharel (Bhattarai), B. Niroula and S. Jha. 2017. Use of the greater duckweed as feed for the tilapia fish. *Nepalese Journal of Biosciences* **7** (1): 117 - 119.

DUCKWEED : THE HIDDEN TREASURE

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ABSTRACT

In recent years, the tiny aquatic angiospermic plants 'duckweeds' have become prominent because they provide high protein animal feed, organic fertilizer, bio-fuel; control mosquitoes; and, have great applicability in wastewater purification, toxicity testing, and in basic research and evolutionary model system. In the aforesaid context, this presentation deals in brief with general characteristics, distribution, environmental requirements, aquaculture, and some uses of duckweeds.

Key words: Productivity, nutritive value, wastewater treatment

INTRODUCTION

Duckweeds are the smallest, fastest growing, and the simplest flowering aquatic plants which float on or just below the surface of nutrient-rich still or slow moving bodies of fresh and slightly brackish waters. They are monocotyledons belonging to the family Lemnaceae, although they are also classified as the subfamily Lemnoideae within the family Araceae (Sheh – May *et al.* 2004). Duckweed consists of five genera: *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*. However, the most commonly available species belong to the three genera *Lemna*, *Spirodela* and *Wolffia* (Table 1, Figs. 1 and 2). The species of Lemnaceae are found in all possible combinations with each other and other floating plants.

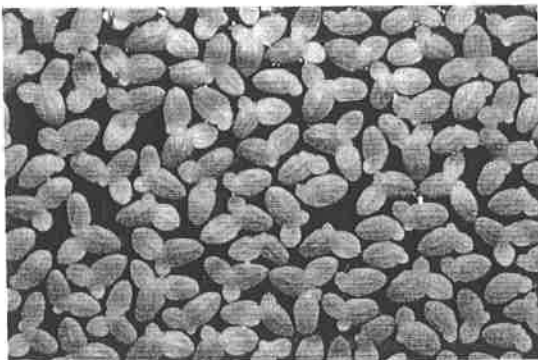


Fig 1. *Lemna aequinoctialis*

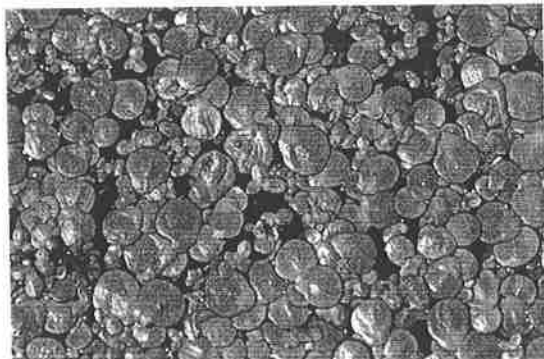


Fig 2. *Spirodela polyrhiza*

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Table 1. Duckweeds of the South Asia [modified from Cook (1996)]

Species	FronD	Root	Seed	Habitat and distribution
<i>Lemna acuinotialis</i> Welwitsch	Flattened (leaf – like), 1 – 6.5 mm long, 0.8 – 4.5 mm wide, floating	Solitary, up to 3.5 cm long, root cap sharply pointed	One per fruit, 0.45 – 0.8 mm long	Eutrophic water bodies like roadside pools, ditches, village ponds, paddy fields, sluggish canals, etc.; often grow together with <i>Spirodela</i> and/or <i>Wolffia</i> ; Pak, Nep, Ban, Mya, SL, Ind (AN, AP, AS, BH, DL, GJ, HP, JH, JK, KL, KT, MH, PJ, RJ, UK, UP, WB)
<i>Lemna gibba</i> L.	Flattened, 4 mm thick, 1-8 mm long, 0.8 – 6 mm wide, floating	Solitary, >3 mm long, root cap rounded	1 – 5 per fruit, 0.7 – 0.9 mm long	Mesotrophic to eutrophic water; Pak, Ind (GJ, JK, PJ)
<i>Lemna minor</i> L.	Flattened, 1-8 mm long, 0.6 – 5 mm wide, floating	Solitary, >3 mm long, root cap rounded	One per fruit, 0.7 – 1 mm long	Mesotrophic to eutrophic water; cooler regions of Pak, Nep, Ind (HP, JK, SK, UK)
<i>Lemna tenera</i> S. Kurz	Flattened, 3.5 – 9 mm long, 1.2 – 3 mm wide, submerged	Solitary, 2.5 mm long, root cap rounded	-	Humid warm region of Mya
* <i>Lemna trisulca</i> L.	Flattened, 3 -15 mm long, 1 – 5 mm wide, submerged except when flowering-fruiting	Solitary, 2.5 mm long, root cap pointed	One per fruit, 0.6 – 1 mm long	Mesotrophic cooler water (sheltered between emergent reeds); Pak, Ban, Ind (JK, MN, RJ, UK, WB)
* <i>Lemna turionifera</i> E. Landolt	Flattened, 2 mm thick, 0.8 – 3.5 mm long, 0.8 – 3.5 mm wide, floating	Solitary, > 3 cm long, root cap rounded	One per fruit, 0.5 – 0.8 mm long	Temperate regions; Ind (HP, JK)
<i>Spirodela intermedia</i> W. Koch	-	2 – 5	-	Native of tropical and subtropical South America; introduced in Ind (DL)
* <i>Spirodela polyrhiza</i> (L.) Schleiden	Flattened, 1.5 – 10 mm long, 1.5 – 8 mm wide, floating	7 – 21	0.7 – 1 mm long	Similar to <i>Lemna acuinotialis</i>
<i>Spirodela punctata</i> (G. F. W. Meyer) Thompson	Flattened, 1.5 – 8 mm long, 1 – 5 mm wide, floating	2 - 7	0.8 – 1 mm long	A native from the Southern Hemisphere and East Asia; naturalized in warm regions of Ind (DL, MH, MN, WB)
<i>Wolffia angustata</i> E. Landolt	Boat – shaped, 0.5 – 0.8 mm long, 0.2 – 0.4 mm wide, submerged except when flowering - fruiting	-	0.3 – 0.4 mm long	Eutrophic village ponds; Ind (WB)

<i>Wolffia arrhiza</i> (L.) Horkel	Spherical to ellipsoid, 0.5 – 1.5 mm long, 0.4 – 1.2 mm wide, submerged except when flowering – fruiting	-	0.4 – 0.5 mm long	Cooler water; Ind (JK)
<i>Wolffia globosa</i> (Roxb.) den Hartog et van der Plas	Ellipsoidal, 0.4 – 0.8 mm long, 0.3 – 0.5 mm wide, submerged	-	-	Warm regions; Pak, Nep, Ban, Mya, SL, Ind (AP, AS, BH, CG, DL, GJ, KL, MP, PJ, RJ, TN, UK, UP, WB)
<i>Wolffia microscopica</i> (Grif.) S. Kurz	Upper surface suborbicular and lower surface tapering downwards, 0.4 – 1 mm long, 0.3 – 0.8 mm wide, submerged except when flowering-fruiting	-	0.3 mm long	Endemic to warmer regions; Pak, Ban, Ind (AP, DL, GJ, HR, TN, UP)
<i>Wolffia neglecta</i> E. Landolt	Boat-shaped, 0.6 – 0.9 mm long, 0.4 – 0.6 mm wide, submerged	-	-	Eutrophic water; endemic to Pak, SL, Ind (RJ, WB)
<i>Wolffiella hyalina</i> (Raff. – Del.) Monod	Boat-shaped, 1 – 3 mm long, 0.8 – 2 mm wide, submerged except when flowering-fruiting	-	0.3 – 0.4 mm long	Native of drier regions of Africa; introduced in Ind (Hyd)

* = Turion forming species; Pak = Pakistan, Nep = Nepal, Ban = Bangladesh, Mya = Myanmar, SL = Sri Lanka, Ind = India : AN = Andaman and Nicobar, AP = Andhra Pradesh, AS = Assam, BH = Bihar, CJ = Chhattisgarh, DL = Delhi, GJ = Gujarat, HP = Himachal Pradesh, HR = Haryana, Hya = Hyderabad, JH = Jharkhand, JK = Jammu and Kashmir, KL = Kerala, KT = Karnataka, MH = Maharashtra, MN = Manipur, MP = Madhya Pradesh, PJ = Punjab, RJ = Rajasthan, SK = Sikkim, TN = Tamil Nadu, UK = Uttarakhand, UP = Uttar Pradesh, WB = West Bengal.

These plants are very simple, lacking an obvious stem or leaves. The plant body is a few cells thick thalloid frond without any root (*Wolffia*, *Wolffiella*) or may have one (*Lemna*) or more adventitious roots (*Landoltia*, *Spirodela*) devoid of root hairs. The bulk of the frond is composed of chlorenchymatous cells separated by large intercellular spaces that are filled with air and provide buoyancy. Some cells of *Lemna* and *Spirodela* have needle-like raphides composed of calcium oxalate. The vascular bundles in all duckweeds are greatly reduced. The upper epidermis in free-floating duckweeds is highly cutinized and is unwettable. Stomata are on the upper side in all five genera. Anthocyanin pigments similar to that in *Azolla* also form in a number of species of Lemnaceae. Roots of duckweeds are usually short but this depends on species and environmental

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conditions and vary from a few millimetres up to 14 cm (Leng 1999). Roots either stabilize the plant on the water surface or assist the plant to obtain nutrients where these are in dilute concentrations. They tend to lengthen as mineral nutrients in water are exhausted.

Duckweeds multiply principally through vegetative propagation by the formation of daughter fronds. New or daughter fronds are produced alternatively from two budding pouches on each side of the mother frond in *Spirodela* and *Lemna*. In *Wolffia* and *Wolffiella* only one budding pouch exists. These budding pouches are situated in *Spirodela* or *Lemna* close to where the root arise, whereas in *Wolffia* and *Wolffiella* the solitary budding pouch is located on the narrow end of the mother frond. Newly formed fronds remain attached to the mother frond during the initial growth phase and the plants therefore appear to consist of several fronds. An individual frond may produce as many as 20 daughter fronds during its lifetime, which lasts for a period of 10 days to several weeks. Generally, however, after six deliveries of daughter fronds, the mother frond tends to die. Duckweed colonies produced in laboratory or naturally are always spotted with brown dead mother fronds. The daughter frond repeats the history of its mother frond.

Vegetative growth in *Lemna minor* exhibits cycles of senescence and rejuvenation, mediated by chemicals released by the mother frond, under constant nutrient availability and consistent climatic conditions (Ashbey and Wangermann 1949). Fronds of *Lemna* have a definite life span, during which, a set number of daughter fronds are produced; each of these daughter fronds is of smaller mass than the one preceding it and its life span is reduced. The size reduction is due to a change in cell numbers. Late daughter fronds also produce fewer daughters than early daughters. At the same time as a senescence cycle is occurring an apparent rejuvenation cycle, in which the short lived daughter fronds (with half the life span of the early daughters) produce first daughter fronds that are larger than themselves and their daughter fronds are also larger, and this continues until the largest size is produced and senescence starts again. The cyclic nature of a synchronized duckweed mat (all fronds of the same age) could be over at least one month as the life span of fronds from early to late daughters can be 33 or 19 days, respectively with a three-fold difference in frond rate production (Wangermann and Ashbey 1950).

When the aquatic ecosystem dries out or declining temperatures occur, duckweeds have mechanisms to persist until conditions return that can support growth. This occurs through late summer flowering, or the production of turions.

Although sexual reproduction is rare in duckweeds, some species, however, reproduce by producing very small unisexual and monoecious flowers and seeds in an inflorescence. In fact, the flower of the duckweed genus *Wolffia* is the smallest known, measuring merely 0.3 mm long (Landolt 1986). The inflorescence generally consists of one female (pistil) and two male flowers (stamens), but in *Wolffia*, there is one male and one female. The flowers are naked or surrounded by spathe. The fruit is a utricle and the seeds are smooth or ribbed. The seeds are resistant to prolonged desiccation and quickly germinate in favourable conditions.

During the season of short photoperiods or cold nights, the newly developing fronds of several species of duckweed get transformed into small brown to olive green orbicular to reniform dormant bodies, called turions. In comparison to normal fronds, turions have shrunken vacuoles, smaller intercellular space, and abundant starch granules. Because the volume of intercellular space shrinks and starch increases the density of the tissue, the turion can sink to the bottom of the water body where it can survive even if top of water freezes. Turions sprout under the favourable conditions using the stored starch as an energy source to give rise to normal fronds capable of further multiplication. Several species survive at low temperatures without forming turions. During the winter season, their fronds are greatly reduced but remain at the water surface.

DISTRIBUTION

In Lemnaceae, disseminules are complete fronds or seeds, and their dispersal occurs by water movement or through surface adhesion to waterfowls. Duckweeds are adapted to a wide variety of geographic and climatic zones and are distributed throughout the world except in waterless deserts, permanently frozen polar regions, and extremely wet areas with very high precipitation (Landolt 2006). Most species are found in moderate climates of tropical and subtropical zones. The appearances of duckweed species not previously seen in areas of Europe have been attributed to rising water temperature throughout the world from global warming (Wolff and Landolt 1994). Some duckweeds appear to tolerate saline waters but they do not concentrate sodium ions in their growth. The apparent limit for growth appears to be between 0.5 and 2.5% sodium chloride for *Lemna minor* (Leng 1999).

ENVIRONMENTAL REQUIREMENTS

A variety of environmental factors, such as water temperature, pH, nutrient concentration, crowding by overgrowth of the colony, competition from other plants for light and nutrients, etc., control the growth and survivability of duckweeds. Maximum, minimum and optimum requirements of some of the most important environmental parameters (temperature, pH, conductivity, nitrogen and phosphorus) are given in Table 2, whereas a range of other important mineral levels found in water supporting Lemnaceae is presented in Table 3.

Table 2. The most important environmental requirements of duckweed (Hasan and Chakrabarti 2009)

Parameters	Minimum	Maximum	Optimum
Temperature (^o C)	> 0	35	15 – 30
pH	3.0	10.0	6.5 – 8.0
Conductivity (μ S/cm)	200	1090	-
Nitrogen (mg/l NH ₄ -N)	Trace	375	7 – 12
Phosphorus (mg/l PO ₄ -P)	0.017	154	4 – 8

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Table 3. Range of other important mineral contents (mg/l) of water supporting Lemnaceae [modified from Landolt (1986)]

Parameters	Absolute range	Range of 95% of the samples
K	0.5 – 100	1.0 – 30
Ca	0.1 – 365	1.0 – 80
Mg	0.1 – 230	0.5 – 50
Na	1.3 - > 1000	2.5 – 300
HCO ₃	8 – 500	10.0 – 200
Cl	0.1 – 4650	1.0 – 2000
S	0.03 – 350	1.0 – 200

The effect of temperature on duckweed growth is affected by light intensity, i.e., as light increases, growth rates increase from 10 to 30°C. Optimum temperature for maximum growth of most duckweed species lies between 17.5 and 30°C (Culley *et al.* 1981, Gaigher and Short 1986). Although some species can tolerate near freezing temperatures, growth rate declines at low temperature. Below 17°C some duckweeds show a decreasing rate of growth (Culley *et al.* 1981). Most species seem to die if the water temperature rises above 35°C.

In general, water pH for aquatic plants is considered excellent between the range 6.5 – 7.5; good between 6 – 6.4 and 7.6 – 8; fair between 5.5 – 5.9 and 8.1 – 8.5; and poor when it is less than 5.5 or more than 8.6 (Stapp and Mitchell 1995). Koirala *et al.* (2011) observed that *S. Polyrhiza* colony survived in pH range of 4 – 7 only when grown alone, but had 2 – 10 range of pH tolerance when grown with *L. aequinoctialis*. Although duckweed survives at pHs between 2 and 10, it grows best only over the range of 6.5 – 8. In this pH range ammonia in water is present largely as the ammonium ion which is the most readily absorbed N form. An alkaline pH (i.e., pH above 8) shifts the ammonium-ammonia balance toward the unionized state and results in the liberation of free ammonia, which is toxic to duckweed at high concentrations (100 mg NH₃/l).

Electrolyte conductivity gives an account of accumulation of salts (primarily chlorides and sulphates of calcium, magnesium, sodium and potassium) in water. Upadhyay *et al.* (2011) reported electrolyte range 24.8 – 184 µS/cm for the occurrence of *L. aequinoctialis* and *S. polyrhiza* in roadside pools at Biratnagar, Nepal. Zutshi and Vass (1973) found *L. gibba* and *L. minor* growing in stagnant waters rich in electrolyte ranging from 400-500 µS/cm. Gopal and Chamanlal (1991) reported the maximum biomass of *L. perpusilla* and *S. polyrhiza* from roadside pools and ditches within a electrolyte conductivity range of 650 – 1000 µS/cm. Khondker *et al.* (1993) recorded the complete disappearance of *S. polyrhiza* by the end of May when a sharp fall in conductivity and alkalinity was observed. The electrolyte conductivity of water supporting the growth of *L. perpusilla* in Bangladesh reported by Islam and Khondker (1991) and Khondker *et al.* (1994) were 625 µS/cm and 200 – 890 µS/cm, respectively. High electrolyte conductivity (1090 µS/cm) of water supporting the growth of *L. perpusilla* was also reported by Van der Does and Klink (1991) in a lemniid habitat in the Netherlands.

One of the most important factors influencing the distribution of aquatic plants is nutrient availability (Hutchinson 1975). Edwards *et al.* (1992) observed that pond water with less than 3

mg/l total kjeldahl nitrogen (TKN) and 0.3 mg/l total phosphorus did not support normal growth of *L. perpusilla* and *S. polyrhiza*. The reason for the comparatively high nutrient demand of free-floating duckweeds resides in the fact that the nutrients are absorbed by the lower surface of the fronds which are rather small compared to that of the root hairs of other plants (Landolt and Kandeler 1987).

The value of duckweed as a feed resource for domestic animals depends on its crude protein content which seems to increase to a maximum of nearly 40 % dry matter over the range from trace ammonia concentrations to 7-12 mg N/l (Leng *et al.* 1994). Culley *et al.* (1981) reported that the TKN of water should not drop below 20 -30 mg/l if the optimum production and a high crude protein content of duckweed are to be maintained. A useful indicator of whether conditions in the pond are appropriate for growth of duckweed (*Lemna spp.*) of high protein content is the length of the roots as there is a close negative relationship between root length and protein content of the duckweed and with the N content of the water. By monitoring this characteristics, the user can have an indication of the nutritive corrective measures when the length of the roots exceeds about 10 mm (Leng 1999).

Duckweeds prefer ammonium nitrogen ($\text{NH}_4\text{-N}$) as a source of nitrogen and will remove it preferentially, even in the presence of relatively high nitrate concentrations. Luond (1980) demonstrated that higher growth rates were attained when nitrogen was in the $\text{NH}_4\text{-N}$ rather than the $\text{NO}_3\text{-N}$ form. In organically enriched waters, nitrogen tends to be concentrated in the $\text{NH}_4\text{-N}$ rather than the $\text{NO}_3\text{-N}$ form at pH levels below 9 and plant growth is equally efficient in anaerobic and aerobic waters (Said *et al.* 1979). In lagoons receiving organic animal wastes, the pH seldom exceeds 8, particularly with a full duckweed cover that suppresses phytoplankton growth (Culley *et al.* 1978). Urea is a suitable fertilizer and is rapidly converted to $\text{NH}_4\text{-N}$ under normal conditions.

Phosphorus is essential for rapid growth and is a major limiting nutrient after nitrogen, although its quantitative requirement for maximum growth is generally low. Fast growing duckweed in nutrient rich water is a highly efficient sink for both phosphorus and potassium; little of each, however, is required for rapid growth. Duckweeds appear to concentrate P up to about 1.5 % of their dry weight and as such are able to grow on high P waters provided the N concentrations are maintained. The plant also appears to be able to draw on the pool of P in its biomass for its biochemical activities and once P had been accumulated it will continue to grow on waters devoid of P. Saturation of phosphate uptake by duckweed occurs at available $\text{PO}_4\text{-P}$ concentrations of 4 to 8 mg/l. Rejmankova (1975) reported good growth of duckweed within the P concentrations of 6 to 154 mg/l. Culley *et al.* (1978), working in dairy waste lagoons, achieved doubled production from 2 to 4 days at P concentrations in excess of 35 mg/l. Reduced growth in some species occurs only when P values drop below 0.017 mg/l (Luond 1980).

Although vigorously growing duckweed is a highly efficient K sink, only low concentrations of K in water are needed to support good growth when other mineral requirements are satisfied. Most decaying plant materials would easily produce the K requirements of duckweed.

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Most research on nutrient requirements have centred on the need for N, P and K. However, like all plants, duckweeds need an array of trace elements and have well developed mechanisms for concentrating these from dilute sources. From the experience of the Non-Government Organization PRISM (Project in Agriculture, Rural Industry, Science and Medicine) in Bangladesh, it appears that providing trace minerals through the application of crude sea salt (9 kg/ ha/day) is sufficient to ensure good growth rates of duckweeds in ponded systems (Leng 1999).

AQUACULTURE

Unproductive marginal land along roads and paths or derelict ponds may be suitable choice to cultivate duckweed, as rental or purchase prices for such lands are usually lower than for arable soil. Additional land for fish ponds is necessary in the case of integrated duckweed-fish production in two-pond systems. The required duckweed/ fish pond area ratios of 1:1 to 2:1 are reported to provide enough duckweed for fish production (Iqbal 1999). Long narrow ponds with water depth between 20 and 50 cm are generally recommended to buffer heat, nutrient and pH extremes by dilution, and to facilitate harvesting (Gaigher and Short 1986). Duckweeds are prone to be blown into heaps by heavy winds or wave action. This allows light to penetrate the water column and would stimulate phytoplankton and algal growth. If the plants become piled up in deep layers, however, the lowest layer will be cut off from light and will eventually die (Skillicorn *et al.* 1993). Plants pushed from the water onto a bank will also dry out and die. To counteract this problem, ponds should be sited perpendicular to the common wind. In large ponds and wide canals a floating bamboo or plastic containment grid system is required to prevent the plants from drifting to the shore by the action of wind or water current. The sides of the ponds must preferably be vertical to prevent the plants from becoming stranded and at least 10 cm higher than the water level to accommodate heavy rains. Banana, papaya, lemon, bamboo, etc., planted on the pond embankments can serve as a protection for duckweed from wind and direct sunlight. Besides, the co-crops may generate additional income.

Rainwater collected in ponds may need a balanced NPK application which can be given as inorganic fertilizer or as rotting biomass, manure or polluted water from agriculture, sewage or industry. The ponds must be fed with effluent through furrows rather than pipes because the latter tend to become clogged. Several inlets must be provided to spread the inflowing nutrients over the pond. Urea is a suitable fertilizer, containing approximately 45 % nitrogen, and is rapidly converted to ammonia under normal conditions. Muriate of potash (MP) and triple superphosphate (TSP) each in a ratio to urea of 1 : 5 work satisfactorily as sources of potassium and phosphorus, whereas crude sea salt is used as the source of trace minerals (Skillicorn *et al.* 1993). Application methods of the inorganic fertilizer include broadcasting, dissolving in the water column of the plot, and spraying a fertilizer solution on the duckweed mat.

A fertilizer application matrix aimed to achieve variable daily production ranging from 500 – 1000 kg of fresh duckweed per hectare was developed by Project in Agriculture, Rural Industry, Science and Medicine (PRISM, an NGO) in their experimental programme at Mirzapur, Bangladesh (Table 4). Furthermore, PRISM recommended daily fertilization rates for different types of duckweed (Table 5). The application rate varies from 21 – 28 kg/ha/day (amounting to >7 tonnes/ ha/ year) with an anticipated fresh biomass yield of 900 – 1000 kg/ ha/ day. The daily fertilization rate for duckweed cultivation developed by the Bangladesh Fisheries Research Institute (B F R I) is presented in Table 6. The fertilizer schedules developed by PRISM and BFRI are very similar (Tables 5 and 6), except that BFRI recommended half the dosage of inorganic fertilizer when cow dung was used at the rate of 750 kg/ ha/ day.

Table 4. Daily fertilizer application matrix for duckweed cultivation (Skillicorn *et al.* 1993)

Fertilizer Application (kg/ ha)	Daily production of fresh plants (kg/ ha)					
	500	600	700	800	900	1000
Urea	10.0	12.0	14.0	16.0	18.0	20.0
TSP	2.0	2.4	2.8	3.2	3.6	4.0
MP	2.0	2.4	2.8	3.2	3.6	4.0
Sea salt	4.5	5.4	6.3	7.2	8.1	9.0

Table 5. Rates of fertilizer application for duckweed cultivation (D W R P 1998)

Duckweed	Rate of application (kg/ ha/ day)		
	Urea	TSP	MP
<i>Spirodela</i>	20	4	4
<i>Wolffia</i>	15	3	4
<i>Lemna</i>	15	3	3

Table 6. Rates of fertilizer application for duckweed cultivation (B F R I 1997)

Fertilizer combination	Rate of application (kg/ ha/ day)			
	Urea	TSP	MP	Cow dung
Inorganic fertilizer only	15 – 20	3 – 4	3 – 4	-
Combination of organic and inorganic fertilizer	7.5	1.5	1.5	750

Any waste organic material that is readily biodegradable and has a sufficiently high nutrient content (Table 7) could be used for duckweed cultivation. The most economical sources of such waste materials are all kinds of animal manure, kitchen wastes, wastes from a wide range of food processing plants, biogas effluents, and slaughter house wastes. Solid materials, such as manure from livestock, night soil from villages, or food processing wastes, can also be mixed with water and added to ponds at suitable levels. All wastewater containing manure or night soil must undergo an initial treatment by holding it for a few days in an anaerobic pond, before using it to cultivate duckweed.

Table 7. Moisture, organic and mineral content of some organic wastes expressed in percent dry matter (Gijzen and Khondker 1997)

Nutrient source	Moisture	Dry organic matter	C	N	P ₂ O ₅	K ₂ O	CaO
Human faecal matter	65 – 80	88 – 97	40.55	5 – 7	3 – 5.5	1 – 2.5	4 – 5
Human urine	93 – 96	65 – 85	11 – 17	15 – 19	2.5 – 5	3 – 4.5	4.5 – 6
Urban refuse	10 – 60	25 – 35	12 – 17	0.4 – 0.8	0.2 – 0.5	0.8 – 1.5	4 – 7.5
Water hyacinth compost	85 – 95	-	-	1.9	1	2.9	4.6
Cow dung (fresh)	85	-	-	0.4	0.02	0.1	-
Cow dung (compost)	-	-	-	0.5	0.3	0.2	0.3
Pig manure (fresh)	80	-	-	0.5	0.5	0.45	-
Poultry (fresh)	-	-	-	1.6	1.5	0.85	-
Digester effluent charged with pig manure	-	6.5	-	3.4	-	-	-

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Sutton and Ornes (1975) and Said *et al.* (1979) demonstrated the necessity of periodic additions of nutrients to small duckweed culture systems receiving municipal or dairy cattle wastes. Within 1-3 weeks, there was a noticeable drop in N, P and K within the plants. There was a corresponding drop in crude protein as the plant nitrogen declined. In fact, due to the high nitrogen requirement of duckweed and the relative rapid loss of nitrogen from aquatic system, this nutrient tend to be limiting in ponds fed with wastewater (Gaigher and Short 1986). Large scale duckweed production therefore requires the availability of relatively large quantities of organic waste.

Algal blooms : Light penetration in the water column and subsequent competition for nutrients and space by algae can become a nuisance when the duckweed mat is incomplete due to disturbances or poor growth. Edwards *et al.* (1987) reported that the filamentous green alga *Spirogyra* bloomed in duckweed ponds fed with latrine effluent. The farmers removed the algae manually, but the algae grew rapidly, became entangled with the duckweed roots and the duckweed fronds turned in colour from green to yellow. In several ponds, duckweed stopped growing and died. Although the ponds were cleaned from dead duckweed and algae and restocked with healthy duckweed, algal blooms reoccurred in most cases.

In another study (Edwards *et al.* 1992), algal blooms of both filamentous algae (mostly the blue-green alga *Oscillatoria* and the green alga *Oedogonium*) and phytoplankton (mostly the blue-green alga *Microcystis*) were reported as one of the most important factors constraining growth of duckweed with septage. The former was more harmful to duckweed as it clogged and wrapped itself around plant roots, causing the fronds of duckweed to shrivel and finally die. Attempts were made to kill algae by the algicide copper sulphate at a concentration of 2 mg/l. Algal growth was inhibited, but duckweed turned yellowish in colour. By changing the harvesting strategy, to maintain an almost complete duckweed cover on the pond surface, algal blooms did not reoccur. However, when algal infestation became severe, it was necessary to clear the pond and restock it with fresh duckweed.

Insect and fungal infestation : Though duckweed growth is reported to be less sensitive to pests and diseases compared to most other aquatic plants (Dinges 1982), insect infestation can cause severe damage and even death of the plants. Fungal infestation inhibits growth.

A study in Thailand revealed that occasional insect infestation by larvae of *Nymphula* (Order Lepidoptera, Family Pyralidae) and/ or by the waterlily aphid *Rhopalosiphum nymphaeae* (Order Homoptera, Family Aphididae) caused heavy damage to duckweed (Edwards *et al.* 1987). Infestation by *Nymphula* was more frequent than by aphids. In one case, *Nymphula* infestation caused the death of plants within two weeks. In the same study, fungal infestation occurred in many ponds and inhibited the growth of duckweed. The fungal infestation resulted in a leaf spot disease and was probably caused by *Mylothecium*, which is also a parasite of the aquatic mosquito fern, *Azolla*. However, farmers, who commercially cultivated duckweed in Taiwan, reported that insects cause no problems to the crops and regarded insect damage as unimportant (Edwards *et al.* 1987).

Application of biocides to control insect and fungal infestation of duckweed is critical due to their extremely high and rapid uptake by duckweed and possible transfer into the food chain. In this context, Zirschky and Reed (1998) opined that a mixture of several duckweed species would be less susceptible to infestations and diseases than a monoculture.

Relief of heat stress : As aforementioned, duckweed growth rapidly declines at temperatures above 31 to 35°C, as the plants experience severe heat stress. Relief of heat stress during extremely hot days can be achieved by manual dunking (dipping the duckweed below the water surface) once a day, which is an efficient and immediate way of lowering temperatures by 5 to 10°C. Dunking consists of agitating the whole-cultivated area by hand until all plants have been physically immersed and wetted.

Productivity : Duckweed growth is largely a function of available nutrients, temperature, light, and degree of crowding. The highest growth rate reported for Lemnaceae under optimal laboratory conditions is about 0.66 generations per day, which corresponds to a doubling time of 16 hours (D W R P 1997). Duckweed generally double their mass in 16 hours to 2 days under optimal nutrient availability, sunlight, and water temperature. This results in an exponential growth, at least until the plants become crowded or run out of nutrients. The rate of harvesting duckweed is important since there is a minimum biomass at which yields will decrease and an upper biomass where yield will be limited by crowding, all other variables being equal. In a study where most of the conditions for growth were unlimited, the effect of harvesting indicated that above about 1.2 kg/m² duckweed (fresh) growth decreased and below 0.6 kg/m² duckweed (fresh) biomass limited growth potential. It appeared that if 1 kg (fresh) duckweed/ m² could be maintained by frequent harvesting then an extrapolated yield of 32 tonnes dry matter/ ha/ year could be produced under other non-limiting conditions (Leng *et al.* 1994). In an experimental programme in Bangladesh, a base *Spirodela* density of 600 g/m² was shown to yield incremental growth of 50 to 150 g/ m² / day (Skillicorn *et al.* 1993). Culley and Myers (1980) obtained an annual dry weight production of 23.31 tonnes/ ha with daily harvesting ranging from 10 to 35 % of the standing crop each day, depending on the season. Edwards (1990) recommended 25 % harvesting of the duckweed biomass at 1 – 3 days intervals when duckweed growth completely covers the pond, with the remaining 75 % left in the pond for further growth.

Table 8 represents the yields of various duckweed species under different environmental conditions. The value varied widely, ranging from 9 to 38 tonnes dry matter/ ha / year. This wide range of productivity may be attributed to differences in species, climatic conditions, nutrient supply and environmental conditions. Many of the reported high yields are based on extrapolated data obtained from short – term growth from small – scale experimental systems rather than potential long – term yields from commercial – sized systems. Edwards (1990) reported extrapolated yields of about 20 tonnes dry matter/ ha / year of *Spirodela* from experiments that were carried out for periods of 1 – 3 months in septage – fed 200 m² ponds in Thailand; however, the yield declined to the equivalent of about 9 tonnes dry matter/ ha / year over a 6 months period. On the basis of available data (Table 8), Hasan and Chakrabarti (2009) opined that an

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average annual yield of around 10 - 20 tonnes dry matter/ ha / year can be obtained from an aquatic environment where nutrients are generally not limiting and frequent harvesting is practised to avoid plant overcrowding.

Table 8. Yields of various duckweed species under different environmental conditions

Species	Environmental condition	Yield (dry matter tonnes/ ha/ year)	Reference
<i>L. minor</i>	Upflow Anaerobic Sludge Blanket Reactor (UASB) effluent	10.7	Vroon and Weller (1995)
<i>L. minor</i>	Nutrient non-limiting water	16.1	Reddy and DeBusk (1984)
<i>L. perpusilla</i>	Septage – fed pond	11.2	Edwards <i>et al.</i> (1990)
<i>L. perpusilla</i> , <i>S. polyrhiza</i> , and <i>W. arrhiza</i>	Septage from septic tank	9.2 – 21.4	Edwards <i>et al.</i> (1992)
<i>Lemna</i>	Domestic wastewater	26.9	Zirschky and Reed (1998)
<i>Lemna</i>	Sugar – mill effluent	32.1	Ogburn and Ogburn (1994)
<i>Lemna</i> , <i>Spirodela</i> and <i>Wolffia</i>	Domestic wastewater	13 – 38	Skillicorn <i>et al.</i> (1993)
<i>Lemna</i> and <i>Wolffia</i>	Faecally polluted surface water	14 – 16	Edwards (1987)
<i>S. polyrhiza</i>	Domestic waste water	17 – 32	Alaerts <i>et al.</i> (1996)
<i>S. polyrhiza</i>	Sewage effluent	14.6	Sutton and Ornes (1975)
<i>S. polyrhiza</i>	Nutrient non-limiting water	11.3	Reddy and DeBusk (1985)

Harvesting and storage : For shallow ponds, the most simple harvesting techniques include manual skimming of the plants from the pond surface with a net, or moving the floating plants to one corner of the pond with a bamboo pole and removing them with baskets. Two people were reported to require 3.5 hours for manual harvesting of duckweed from a 0.3 ha pond in Taiwan (Iqbal 1999). Large – scale harvesting in industrialized countries is carried out with mechanical harvesting machines requiring, however, deep ponds.

High moisture content of the fresh duckweed increases its handling, transport and drying costs. This fact is less important in integrated systems where fresh duckweed is fed to animals as the only feed or, in combination with other feed components. Fresh duckweed can be stored temporarily in a cool, humid place, such as in a small tank or pool. The fresh material, which will begin to ferment at high temperatures after a few hours, can be preserved for several days if kept cool and damp (Skillicorn *et al.* 1993).

The economic potential of duckweeds may not be fully realized until it can be economically reduced to a dried, compact commodity. This requires solar drying and either pelleting, powdering or other potential preservation methods like ensilaging. The waxy coating on the upper surface of duckweed plants is a good binding agent for pelleting. It can be stored for five or more years in the form of dried pellets. Sealable, opaque plastic bags are recommended for long-term storage to protect dried pellets from humidity, insects, vermin, and direct sunlight (Skillicorn *et al.* 1993).

Nutritive value : The entire body of duckweeds is composed of non-structural, metabolically active tissue; most photosynthesis is devoted to the production of protein and nucleic acids, making the plants very high in nutritive value. The nutritional content of duckweed is probably more dependent on the mineral

concentrations of the growth medium than on the species or their geographic location (Hasan and Chakrabarti 2009). Water low in nutrients generally results in reduced nutritional content and slow growth in duckweeds.

Compared with most plants, duckweed fronds have little fibre as they do not need to support upright structures. Crude fibre content is generally lower (varying between 7 – 10 %) for duckweeds grown in nutrient-rich water than that grown in nutrient-poor water (11 – 17 %) (Leng *et al.* 1994). Similarly, ash content (that ranges between 12 – 18% in duckweeds) is also higher in duckweed colonies with slow growth.

Duckweeds are rich source of nitrogen, phosphorus, potassium and calcium (Guha 1997). The concentration of N and P in duckweed tissues depend on the amount of N and P in the water, up to a threshold concentration that has not been clearly defined. Above this threshold, there is little increase in the tissue.

When conditions are good, duckweed contains considerable protein, fat, starch and minerals, which appear to be mobilized for biomass growth when nutrient concentrations in the growth medium fall below the critical levels for growth. The crude protein content of duckweed seems to increase from trace ammonia concentrations to 7 – 12 mg N/l when crude protein reaches a maximum of about 40 % (Leng *et al.* 1994). Assuming a mean annual yield of 17.6 tonne dry matter/ ha / year, with a protein content of 37 % dry weight, a protein production of about 6.5 tonne/ ha / year can be obtained. This per hectare protein yield is far higher than for most other crop plants, and about 10 times that of soyabean (Table 9). This remarkable value for duckweed is not only attributed to its high growth rate and high protein content, but also to the fact that the entire biomass of duckweed is used as compared to only the seeds for most crops (Gijzen and Khondker 1997). Besides, duckweed protein has a better amino acid profile than most plant proteins and more closely resembles animal protein than any other plant proteins. The levels of amino acids are very similar in the various species and all the essential amino acids are generally present.

Table 9. Comparison of protein yields of duckweed and selected crops (Gijzen and Khondker 1997)

Plant/ Crop	Yield (tonne dry matter/ ha/ year)	Crude protein (% dry weight)	Relative protein production*
Duckweed	17.6	37	100
Soyabean	1.59	41.7	10.2
Alfalfa hay	4.37 – 15.69	15.9 – 17	11.4 – 38.3
Peanuts	1.6 – 3.12	23.6	5.7 – 11.3
Cottonseed	0.76	24.9	2.9

* Relative protein production: duckweed set at 100 units = 6.51 tonne dry matter/ ha/ year

The lipid content is lower (1.8 – 2.5 %) in duckweed species grown in nutrient – poor water, while it generally varies between 3 – 7 % for duckweed grown in nutrient – rich water (Hasan and Chakrabarti 2009). Cultured duckweed has high concentrations of trace minerals and pigments, especially β -carotene and xanthophylls (Haustein *et al.* 1988). Duckweeds, however, store varying

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amounts of calcium as calcium oxalate crystals in the vacuoles. A summary of the nutritional composition of different species grown under different environmental conditions is presented in Table 10.

USE OF BIOMASS

Human consumption : *Wolffia arrhiza* has traditionally been eaten in Myanmar, Laos, and northern Thailand (Bhanthumnavin and McCarry 1971). However, the use of Lemnaceae for human consumption has surprisingly not spread to other regions of the world. A possible explanation could be its high content of crystallized oxalic acid which has a negative effect on the taste. Another factor contributing to the low interest in duckweed as a potential food product for human consumption could be attributed to the fact that it is difficult to separate associated (pathogenic) organisms such as worms, snails, protozoa, and bacteria from the plant (Gijzen and Khondker 1997).

Animal feed : Use of duckweed as fish feed is by far the most widespread application. Duckweeds can be fed to fish in fresh form as a sole feed or in combination with other feed ingredients. Duckweeds are also fed as a dried meal ingredient in pelleted diets. Fresh and dried duckweed are fed to grass carp, Nile tilapia, common carp, Indian major carps (rohu and mrigal), silver carp, Java barb, hybrid grass carp and hybrid tilapia.

Table 10. Chemical analyses of various duckweed species grown under different environment conditions

Duckweed species	Aquatic environment	Moist ure (%)	Proximate composition ¹ (% dry matter)					Minerals (% dry matter)		Reference
			CP	EE	Ash	CF	NFE	Ca	P	
<i>L. aequinoctialis</i> , Nepal	Low-nutrient ² roadside pool	-	7.1	-	-	-	-	-	0.35	Koirala (2015)
<i>L. gibba</i> , USA	Low-nutrient ² lagoon	-	9.4	1.8	16.8	17.0	55.5	1.38	0.72	Culley et al. (1981)
<i>L. minor</i> , Bangladesh	Pond, nutrient status not specified	92.0	14.0	1.9	12.1	11.1	60.9	-	-	Zaher et al. (1995)
<i>L. minor</i> , Bangladesh	Ditch, nutrient status not specified	93.8	20.3-23.5	-	-	-	-	-	-	Majid et al. (1992)
<i>S. polyrhiza</i> , USA	Low-nutrient ² lagoon	-	13.1	2.5	13.3	16.1	55.0	1.21	0.56	Culley et al. (1981)
<i>S. polyrhiza</i> , Bangladesh	Ditch, nutrient status not specified	95.0	17.3-28.4	-	-	-	-	-	-	Majid et al. (1992)
<i>S. polyrhiza</i> , Nepal	Low-nutrient ² roadside pool	-	13.3	-	-	-	-	-	0.32	Koirala (2015)
<i>S. punctata</i> , USA	Low-nutrient ² lagoon	-	10.6	2.3	14.1	11.3	61.7	0.98	0.61	Culley et al. (1981)
<i>W. arrhiza</i> , Bangladesh	Ditch, nutrient status not	91.2	14.9	-	-	-	-	-	-	Majid et al. (1992)

<i>L. gibba</i> , USA	specified High-nutrient lagoon ³	-	36.3	6.3	15.5	10.1	31.8	1.81	2.60	Culley et al. (1981)
<i>L. gibba</i> , USA	Dairy cattle waste lagoon	-	38.5	3.0	16.4	9.4	32.7	1.00	1.60	Hillman and Culley (1978)
<i>L. minima</i> , USA	Source not specified	-	31.0	2.0	14.0	10.0	42.2	-	-	Shireman et al. (1977)
<i>L. perpusilla</i> , Thailand	Septage-fed earthen pond	94 - 94.3	25.3- 29.3	3.8- 4.5	15.4- 17.6	6.9-7.6	-	-	-	Hassan and Edwards (1992)
<i>S. oligorrhiza</i> , USA	Dairy cattle waste lagoon	-	37.8	3.8	12.0	7.3	39.1	1.30	1.50	Hillman and Culley (1978)
<i>S. oligorrhiza</i> , USA	Treated waste water effluent	-	32.7	6.3	20.3	13.5	27.2	1.49	1.15	Culley and Epps (1973)
<i>S. oligorrhiza</i> , USA	Anaerobic swine waste lagoon	-	41.4	5.1	12.9	8.3	32.3	0.91	2.07	Culley and Epps (1973)
<i>S. polyrhiza</i> , Thailand	Septage-fed earthen pond	91.0	23.8	3.8	18.3	11.7	42.4	-	-	Hassan and Edwards (1992)
<i>S. polyrhiza</i> , USA	High-nutrient lagoon ³	-	39.7	5.3	12.8	9.3	32.9	1.28	2.10	Culley et al. (1981)
<i>S. polyrhiza</i> , USA	Dairy cattle waste lagoon	-	40.9	6.7	12.9	8.7	30.8	2.10	1.40	Hillman and Culley (1978)
<i>S. punctata</i> , USA	High-nutrient lagoon ³	-	36.8	4.8	15.2	9.7	33.5	1.75	1.50	Culley et al. (1981)

¹ CP = Crude protein, EE = ether extract, CF = crude fibre, NFE = nitrogen free extract, Ca = Calcium, P = Phosphorus

² Low-nutrient water body contained less than 5 mg/l TKN

³ High-nutrient water body contained more than 30 mg/l TKN

Fresh duckweeds are fed as a sole feed (*ad libitum* or at restricted level) whereas dried duckweed meal is incorporated by partially replacing other conventional feed ingredients (oil cake, wheat bran, rice bran, etc.) in pelleted diets. *Ad libitum* feeding of fresh duckweed is mostly used for herbivorous fish. Nikolskij and Verigin (1996) reported that grass carp consumed fresh duckweed equal to their body weight over a 24 hour period. Baur and Buck (1980) reported that grass carp consumed from 85 % to 238 % of their body weight/ day (BW/ day) on a mixed diet of *Lemna*, *Spirodela* and *Wolffia* spp. Shireman et al. (1977) recorded consumption rates varying from 7.2 – 7.4 % BW/day on a dry weight basis (DW) while fresh duckweed (*L. minima*) was fed *ad libitum*. Since duckweed contains about 92 % moisture, the dry weight feeding rates given above are equivalent to 90 – 92 % BW/day on a fresh weight basis. Hassan and Edwards (1992) studied the effect of feeding rate of *L. perpusilla* on the survival, growth and food conversion rate of Nile tilapia and recorded that the optimal daily feeding rates of *Lemna* were 5, 4 and 3 % BW/ day DW for fish of 25 - 44 g, 45 – 74 g and 75 – 100 g, respectively. Though carp polyculture using duckweed as the only feed input is reported to be feasible, there is some evidence that duckweed as a sole feed for

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fish is a diet too low in fats and carbohydrates. Therefore, for a balanced diet a mixture of 50 -60 % (DW) duckweed and 40-45 % (DW) fat and carbohydrate-rich feed has been suggested (Iqbal 1999).

Haustein *et al.* (1992) reported a reduced meat production and a lower *feed conversion ratios* (g dry duckweed per g animal fresh weight) for pigs fed on duckweed whose protein content amounted to 23 % and fibre content to 7.5 % dry matter. Galkina *et al.* (1965), however, demonstrated a clearly positive effect on the weight gain of pigs when duckweed was added as a supplement to the normal diet. Further research is needed to show how duckweed can be used as the protein source in diets for pigs. Studies using fresh and dry duckweed and conventional (grain – based) and non-conventional feeds (for example, sugarcane juice or molasses) are urgently needed.

Observations in Bangladesh and Taiwan (Edwards *et al.* 1987) clearly revealed that ducks readily feed on fresh duckweed, often directly from the pond surface. However, chickens are preferably fed on dried duckweed. In general, small amounts (2 – 25 % of total dry matter fed) of duckweed in the diet stimulate the growth of chickens, while higher additions (> 40 %) of duckweed tend to decrease weight gain (Haustein *et al.* 1988). There are reports of an increase in weight by 10-32 % for chicken fed with small amounts of duckweed (2 – 5 %) in addition to their regular diet (Iqbal 1999). Shahjahan *et al.* (1981) obtained very good results with a 10 % addition of *Spirodela* to a mixed chicken diet.

Duckweeds grown on nutrient-rich waters have the potential to be of high nutritional value particularly for the young or lactating ruminant and preliminary observations suggest that they might form the basis of a supplement to diets based on mature biomass such as crop residues, mature grass or pasture. Rusoff *et al.* (1980) reported that up to 75 % of duckweed could be fed to Holstein cattle without affecting the taste of milk. The weight gain of calves fed with a mixture of duckweed (67 %) and silage of corn (33 %) showed a daily weight gain of 0.95 kg, compared to only 0.5 kg weight gain when fed on a concentrate/ corn silage diet. Culley *et al.* (1981) calculated that a 3.1 ha surface area of duckweed cultivation could provide sufficient protein to feed 100 dairy cattle. Taubaev and Abdiev (1973) reported an additional weight gain of up to 27 % and 14 % for ram and sheep, respectively, upon feeding the animals 0.5 kg/day Lemnaceae in addition to their regular diet. However, Leng *et al.* (1994) mentioned that the contribution of duckweed protein in ruminant nutrition is doubtful, as the duckweed protein is readily fermented by microorganisms in the rumen, and the amino acid supply to the animal is, thereby, minimized. It is likely that duckweed is initially used as a source of essential microbial nutrients to enhance the efficient fermentative digestion of straw in the rumen. Nevertheless, feed technology research is needed to enhance the use of duckweed as direct protein source for ruminants.

Organic fertilizer : Duckweed can be used as an organic fertilizer in agriculture by direct land application or via composting. According to Lot *et al.* (1979), application of duckweed eventually contributed to a superior soil texture, including an improved water and cation exchange, and resulted in harvest of 4 crops of vegetables or corn, annually.

Biofuel : As mentioned earlier, there are conditions like temperature shifts due to seasons that can cause a morphological change of the normal fronds of several species of duckweed to a different structure, called turions. Turion formation can also be induced by transferring the fresh fronds from a nutrient-rich medium to tap water (Cheng and Stomp 2009), addition of abscisic acid (ABA) in the growth medium (Perry and Byrne 1969), or by “ hot day – hot night “ treatment of normal fronds of 30^oC photo-temperature and 25^oC dark-temperature and a photoperiod of 16 hours (Perry 1968). Because fronds have little lignin (Blazey and McClure 1968), which would interfere with the digestion of the carbohydrate fraction (which amounts up to 70 % in dry weight) of biomass, and turions have high starch content (60.1% in dry mass), duckweed might also be suitable as an alternative source of bioenergy (Wang and Messing 2012). Whereas cellulose is a crystalline, compact and structural compound resistant to biological attack and enzymatic degradation, starch is readily digested. Even though many advances have been made in the commercialization of cellulosic biomass (Gray *et al.* 2006), the cost of producing equal amounts of ethanol from cellulosic biomass is still much higher than production directly from starch (Wyman 2003). Therefore, growing attention is being devoted to use duckweeds as a source of carbon compounds and convert duckweed biomass into bio-ethanol (Cheng and Stomp 2009).

Mosquito control : A positive effect of a duckweed cover on the decrease of mosquito larvae was reported for *S. punctata* (Furlow and Hays 1972), *L. minor* (Angerilli and Beirne 1980), *Wolffia* (Bentley 1910), and *Spirodela* (Culley and Epps 1973). The authors suggested that a complete duckweed cover acts either as physical barrier and hinders the mosquito larvae from reaching the surface for oxygen uptake, or that the plants release compounds which are toxic to the larvae (Bentley 1910, Judd and Borden 1980). A possibly reducing effect of duckweed on mosquito breeding may positively contribute to the acceptance of duckweed farming systems in areas where mosquitoes are a nuisance and a vector of serious human diseases like malaria or dengue (Iqbal 1999)

WASTEWATER TREATMENT

Solids are relatively easy to remove, what is most difficult to remove from the wastewaters are dissolved salts such as nitrates, phosphates, and other nutrients, and toxic metal ions and organic compounds (xenobiotic pesticides, etc.). In this context, the basic concept of a duckweed wastewater treatment system is to farm local duckweed on the wastewater requiring such treatment. Duckweed systems distinguish themselves from other wastewater treatment mechanisms in that they also produce a valuable, protein-rich biomass as a by-product.

Landolt and Kandeler (1987) reported that of all aquatic plants, duckweeds have the greatest capacity in assimilating the macro-elements N, P, K, Ca, Na and Mg, however, this may not be supported by other literature sources. The data presented in Table 11 suggests that nutrient removal rates for duckweed are comparatively slower than for other aquatic plants and, therefore, longer retention times will be necessary to reduce nutrient concentrations to specific discharge

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limits. Gijzen and Khondker (1997) stated that despite contradictory data, it is an established fact that duckweed has a high nutrient removal efficiency.

Table 11. Daily nitrogen and phosphorus uptake rates by different floating aquatic macrophytes (DeBusk and Reddy 1987, Reddy and DeBusk 1985)

Plant	Uptake (g/ m ² / day)			
	N		P	
	Summer	Winter	Summer	Winter
Water hyacinth	1.30	0.25	0.24	0.05
Water lettuce	0.99	0.26	0.22	0.07
Pennywort	0.37	0.37	0.09	0.08
Duckweed (<i>S. polyrrhiza</i>)	0.15		0.03	

Water hyacinth has been widely used for its extremely high nutrient uptake efficiency (Table 11). However, no economically attractive application of the harvested biomass has so far been identified. In addition, water hyacinth only grows efficiently in tropical climates. A specific comparison of duckweed with water hyacinth for wastewater treatment and biomass use is presented in Table 12.

Table 12. Comparison between duckweed and water hyacinth for wastewater treatment and biomass use

Criterion	Duckweed	Water hyacinth
Tolerance to low temperatures	Higher	Lower, more restricted to warm climates
Nutrient uptake capacity	<ul style="list-style-type: none"> • High, but smaller contact area with the wastewater surface • High tolerance to high nutrient concentrations 	Higher, due to greater contact area with the wastewater through root hairs
BOD removal efficiency	<ul style="list-style-type: none"> • Lower, because of smaller surface area for attached bacteria growth and lower oxygen supply. • Lower tolerance to high BOD concentrations (< 200 mg/l) 	<ul style="list-style-type: none"> • Higher because of larger surface area for attached bacteria growth and higher oxygen supply to the root zone. • Treatment of wastewater with very high BOD concentrations (> 1000 mg/l)
Removal capacity of organic xenobiotics and heavy metals	High	High
Mosquito control	Positive	Negative

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Harvesting	<ul style="list-style-type: none"> • Easier • Can be done manually and mechanically 	<ul style="list-style-type: none"> • Complicated, because plants are bulky and interconnected over large distances • Mechanical harvesting equipment necessary
Nutrient profile (in % dry weight) when grown on wastewater	<ul style="list-style-type: none"> • Protein (30 – 45 %) • Carbohydrate (35 %) • Fibre (7 – 14 %) • Fat (3 – 7 %) • High vitamin and mineral content 	<ul style="list-style-type: none"> • Protein (10 -25 %) • Carbohydrate (35-72 %) • Fibre (17 -20 %) • Fat (1 -3 %)
Use of biomass	<ul style="list-style-type: none"> • High quality feed supplement for fish and other animals • Land application • Composting • Methane and ethanol fermentation • Medicinal plant 	<ul style="list-style-type: none"> • Generally not consumed by fish and other animals • Land application • Composting • Biogas digestion • Paper production
Water loss through evapotranspiration (ET)	Lower ET rates compared to open water (20 – 30 % reduction)	Equal or increased ET rates compared to open water

Duckweeds have been generally applied for the treatment of domestic or agricultural wastewaters. However, they may also be applied for the treatment of wastewaters arising from industries like petroleum, paper manufacturing, metal extraction, etc., which often contain toxic substances, notably, heavy metals (defined as elements with density $>5 \text{ mg/ cm}^3$ such as cadmium, chromium, copper, lead, mercury, zinc, etc.) and a variety of organic compounds. Lemnaceae can tolerate and accumulate high concentrations of heavy metals and organic compounds at accumulation factors ranging multiples of 10^2 and 10^5 (the accumulation factor for heavy metals being much higher at low metal concentrations). It is therefore, important that the plants are harvested at regular intervals to prevent the metals and organic compounds from settling on the sediments with the decaying duckweed. The duckweed cover or sections of it grown on wastewater contaminated with heavy metals and organic toxins should, under no circumstances be used anymore as animal feed or organic fertilizer, but rather be disposed off as safely as possible in bottom-sealed landfills. Alternatively, heavy metals can be regained from the plant tissues through low temperature carbonization.

GENERAL CONSIDERATIONS

Duckweeds, the tiny aquatic plants, have a leaf-like body, called frond that performs photosynthesis. Fronds grow vegetatively and can increase biomass rapidly, lowering carbon dioxide in the air and reducing nitrogen and phosphorus in the water. Duckweeds have been fed to animals and fish to complement diets, largely to provide a protein of high biological value. Besides, the dormant phase of some duckweed species, called turions, is rich in starch, a suitable substrate for ethanol production. However, like a hidden treasure, duckweeds are unutilized in Nepal.

The growing awareness of water pollution and its threat to the ecology of a region and agriculture per se has also focussed attention on potential biological mechanisms for cleansing water of these impurities making it potable and available for reuse. There are thousands of derelict ponds polluted to eutrophication levels in Nepal alone, that could potentially be cleansed of much of their pollutants and resurrected for duckweed aquaculture and fish farming. To resurrect derelict ponds, the approach might be to first establish duckweed aquaculture as a source of nutrients for terrestrial crop production (for example, mulches and organic fertilizer) and as the ponds' oxygen levels rise with harvesting of the crop, to introduce fish farming either in part of the pond or in adjacent (clean water) ponds.

Duckweeds will remain an unutilized/ underutilized resource unless the farmers are familiar with their economic and environmental values. There is vast need for popularization, market, and research support for the duckweed.

REFERENCES

- Alaerts, G. J., Md. M. Rahman and P. Kelderman. 1996. Performance analysis of a full-scale duckweed-covered sewage lagoon. *Water Research* **30(4)** : 843 – 852.
- Angerilli, N. P. D. and B. P. Beirne. 1980. Influence of aquatic plants on colonization of artificial ponds by mosquitoes and their insect predators. *Canadian Entomology* **112** : 793 – 796.
- Ashbey, E. and E. Wangermann. 1949. Senescence and rejuvenation in *Lemna minor*. *Nature* **164** : 187.
- Baur, R. J. and D. H. Buck. 1980. Active research on the use of duckweeds in the culture of grass carp, tilapia, and freshwater prawns. *Illinois Natural History Survey, RRI, Kinmundy*. (polycopy).
- Bentley, C. A. 1910. The natural history of Bombay malaria. *Journal of Bombay Natural History Society* **20** : 392 – 422.
- B F R I . 1997. *Research Progress Report : January – August, 1997*. Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh.
- Bhanthumnavin, K. and M. G. McGarry. 1971. *Wolffia arrhiza* a possible source of inexpensive protein. *Nature* **232** : 495.
- Blazey, E. B. and J. W. McClure. 1968. The distribution and taxonomic significance of lignin in the Lemnaceae. *American Journal of Botany* **55 (10)** : 1240 – 1245.
- Cheng, J. J. and A. M. Stomp. 2009. Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean – Soil, Air, Water* **37 (1)** : 17-26.
- Cook, C. D. K. 1996. *Aquatic and Wetland Plants of India*. Oxford University Press, New Delhi.

Nep J Biosciences

- Culley, D. D. and A. E. Epps. 1973. Use of duckweeds for waste treatment and animal feed. *Journal of the Water Pollution Control Federation* **45** : 337 – 347.
- Culley, D. D. and R. W. Myers. 1973. *Effect of Harvest Rate on Duckweed Yield and Nutrient Extraction in Dairy Waste Lagoons*. US Department of Energy Final Report. School of Forestry and Wildlife Management, Louisiana State University, Baton Rouge.
- Culley, D. D., E. Rejmenkova, J. Kvet and J. B. Frye. 1981. Production, chemical quality and use of duckweeds (*Lemnaceae*) in aquaculture, waste management, and animal feeds. *Journal of the World Mariculture Society* **12 (2)** : 27 – 49.
- Culley, D. D., J. H. Gholson, T. S. Chisholm, L. C. Standifer and E. A. Epps. 1978. *Water Quality Renovation of Animal Waste Lagoons Utilizing Aquatic Plants*. US Environmental protection Agency, Oklahoma.
- DeBusk, T. A. and K. R. Reddy. 1987. BOD removal in floating aquatic macrophyte-based wastewater treatment systems. *Water Science and Technology* **19** : 273 – 279.
- Dinges, R. 1982. *Natural Systems for Water Pollution Control*. Van Nostrand Reinhold, New York.
- D W R P. 1997. *Literature Review 1*. Duckweed Research Project, Ministry of Fisheries and Livestock and DHN Consultants, Dhaka.
- D W R P. 1998. *Duckweed in Bangladesh*. Duckweed Research Project, Ministry of Fisheries and Livestock and Royal Netherlands Embassy, Dhaka.
- Edwards, P. 1987. Use of terrestrial vegetation and aquatic macrophytes in aquaculture. In D. J. W. Moriarty and R. S. V. Pullin (eds.) *Detritus and Microbial Ecology in Aquaculture*. ICLARM Conference Proceedings No. 14, Manila. pp. 311 – 335.
- Edwards, P. 1990. An alternative excreta – reuse strategy for aquaculture: the production of high protein feed. In P. Edwards and R. S. V. Pullin (eds.) *Wastewater – fed Aquaculture*. Environmental Sanitation Information Center, Asian Institute of Technology, Bangkok. pp. 209 – 221.
- Edwards, P., C. Polprasert and K. L. Wee. 1987. *Resource Recovery and Health Aspects of Sanitation*. Asian Institute of Technology Report no. 205, Bangkok.
- Edwards, P., M. S. Hassan, C. H. Chao and C. Pacharaprakiti. 1992. Cultivation of duckweeds in septage – loaded earthen ponds. *Bioresource Technology* **40** : 109 – 117.
- Furlow, B. M. and K. L. Hays. 1972. Some influences of aquatic vegetation on the species and number of Culicidae (Diptera) in small pools of water. *Mosquito News* **32** : 595 – 599.
- Gaigher, I. G. and R. Short. 1986. An evaluation of duckweed (*Lemnaceae*) as a candidate for aquaculture in South Africa. In R. D. Wamsley and J. G. Wan (eds.) *Aquaculture 1980*. Report Series no 15, CSIRO, Canberra. pp. 81 – 90.
- Galkina, N. V., D. A. Abdullaev and V. L. Zacharova. 1965. Biological feed features of duckweeds. *Uzbekistan Biological Journal* **3** : 44 – 47.
- Gijzen, H. J. and M. Khondker. 1997. An overview of the ecology, physiology, cultivation and applications of duckweed. *Inception Report. Annex 1. Literature Review*. Duckweed Research Project, Dhaka.

JHA AND POKHAREL (BHATTARAI) – DUCKWEED : THE HIDDEN TREASURE

- Gopal, B. and Chamanlal. 1991. Distribution of aquatic macrophytes in polluted water bodies and their bio-indicator value. *Verhandlungen des Internationalen Vereins fur Limnologie* **24** : 2125 – 2129.
- Gray, K. A., L. Zhao and M. Emptage. 2006. Bioethanol. *Current Opinion Chemical Biology* **10 (2)** : 141 – 146.
- Guha, R. 1997. Duckweeds. *Envis Newsletter* (March 1997) : 5 – 9 (Indian Institute of Science, Bangalore).
- Hasan, M. R. and R. Chakraborti. 2009. *Use of Algae and Aquatic Macrophytes as Feed in Small-scale Aquaculture : A Review*. Food and Agriculture Organization of the United Nations, Rome.
- Hassan, M. S. and P. Edwards. 1992. Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) as feed for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **104** : 315 – 326.
- Haustein, A. T., R. H. Gillman, P. W. Skillicorn, V. Guevara, F. Dias, V. Vergara, A. Gastanaduy and J. B. Gillman. 1992. Compensatory growth in broiler chicks fed on *Lemna gibba*. *British Journal of Nutrition* **68 (2)** : 329 – 335.
- Haustein, A. T., R. H. Gillman, P. W. Skillicorn, V. Vergara, V. Guevara and A. Gastanaduy. 1988. Duckweed, a useful strategy for feeding chickens: performance of layers fed with sewage – grown *Lemnaceae* species. *Poultry Science* **69** : 1835 – 1844.
- Hillman, W. S. and D. D. Culley. 1978. The uses of duckweed. *American Scientist* **66** : 442 – 451.
- Hutchinson, G. E. 1975. *A Treatise on Limnology; Vol. 3 : Limnological Botany*. John Wiley & Sons, New York.
- Iqbal, S. 1999. *Duckweed Aquaculture: Potentials, Possibilities and Limitations for Combined Wastewater Treatment and Animal Feed Production in Developing Countries*. SANDEC Report No. 6/99. Department of Water & Sanitation in Developing Countries, Swiss Federal Institute for Environmental Science & Technology, Duebendorf.
- Islam, A. K. M. N. and M. Khondker. 1991. Preliminary limnological investigations of some polluted waters covered by duckweeds. *Bangladesh Journal of Botany* **20** : 73–75.
- Judd, G. J. R. and J. H. Borden. 1980. Oviposition deterrents for *Aedes aegypti* in extracts of *Lemna minor*. *Journal of Entomological Society* **77** : 30 – 33.
- Khondker, M., A. K. M. N. Islam and A. D. Makhnun. 1994. *Lemna perpusilla* screening on habitat limnology. *Bangladesh Journal of Botany* **23** : 91 – 106.
- Khondker, M., A. K. M. N. Islam and N. Nahar. 1993. A preliminary study on the growth rate of *Spirodela polyrrhiza*. *Dhaka University Journal of Biological Sciences* **2** : 197 – 200.
- Koirala, U. 2015. *Ecological Study of Wetland Macrophytes Occurring in Morang District of Nepal*. Ph. D. Thesis, Tribhuvan University, Kathmandu.
- Koirala, U., S. Jha and B. Niroula. 2011. Effect of different water pH on growth of some free-floating macrophytes at Biratnagar, Nepal. *Geobios* **38 (2-3)** : 212 – 213.
- Landolt, E. 1986. *The family of Lemnaceae – A monographic Study : Morphology, Karyology, Ecology, Geographic distribution, Systematic position, Nomenclature, Descriptions*, Vol. 2. Veroffentlichungen des Geobotanisches Institute der Eidgenossische Technische Hochschule, Stiftung Ruebel, Zurich.

- Landolt, E. 2006. Duckweed. In *Flora of North America North of Mexico*, Vol. 22. Flora of North America Editorial Committee, New York and Oxford.
- Landolt, E. and R. Kandeler. 1987. *The family of Lemnaceae – A Monographic Study : Phytochemistry, Physiology, Application and Bibliography*, Vol. 4. Veröffentlichungen des Geobotanisches Institut der Eidgenössische Technische Hochschule, Stiftung Ruebel, Zurich.
- Leng, R. A. 1999. *Duckweed : A Tiny Aquatic Plant with Enormous Potential for Agriculture and Environment*. Food and Agriculture Organization of the United Nations, Rome.
- Leng, R. A., J. H. Stambolie and R. Bell. 1994. Duckweed – a potential high-protein feed resource for domestic animals and fish. In *Proceedings of the 7th Animal Science Congress of the Asian – Australasian Association of Animal Production Societies (AAAP) Conference*. Indonesian Society of Animal Science, Jakarta. pp. 103-114.
- Lot, A., A. Novelo and A. Quiroz, 1979. The chinampa : an agricultural system that utilizes aquatic plants. *Journal of Aquatic Plant Management* **17** : 74 – 75.
- Luond, A. 1980. Effects of nitrogen and phosphorus upon the growth of some *Lemnaceae*. In E. Landolt (ed.) *Biosystematic Investigations in the Family of Duckweeds (Lemnaceae)*. Veröffentlichungen des Geobotanisches Institute der Eidgenössische Technische Hochschule, Stiftung Ruebel, Zurich. pp. 118-141.
- Majid, F. Z., R. Khatun, N. Akhtar and A. S. M. A. Rahman. 1992. Aquatic weeds as a source of protein in Bangladesh. *Bangladesh Journal of Scientific and Industrial Research* **27** : 103 – 111.
- Nikolskij, G.V. and B.V. Verigin. 1966. The basic biological characteristics of white amur and bighead and their acclimatization in the water reservoirs of our country. In *Herbivorous Fish*. Piscevaja Promyslennost, Moscow. pp. 30-40.
- Ogburn, D. M. and N. J. Ogburn. 1994. Use of duckweed (*Lemna* sp.) grown in sugarmill effluent for milkfish, *Chanos chanos* Forskal, production. *Aquaculture and Fisheries Management* **25** : 497 – 503.
- Perry, T. O. 1968. Dormancy, turion formation, and germination by different clones of *Spirodela polyrrhiza*. *Plant Physiology* **43** : 1866 – 1869.
- Perry, T. O. and O. R. Byrne. 1969. Turion induction in *Spirodela polyrrhiza* by abscisic acid. *Plant Physiology* **44** : 784 – 785.
- Reddy, K. R. and W. F. DeBusk. 1984. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water : 1. Water hyacinth, water lettuce, and pennywort. *Economic Botany* **38** : 229 – 239.
- Reddy, K. R. and W. F. DeBusk. 1985. *Textbook of Fish Genetics and Biotechnology*. Indian Council of Agricultural Research, New Delhi.
- Rejmankova, E. 1975. Comparison of *Lemna gibba* and *Lemna minor* from the production viewpoint. *Aquatic Botany* **1** : 423 – 427.
- Rusoff, L. L., E. W. Blakeney and D. D. Culley. 1980. Duckweeds (*Lemnaceae* family) : a potential source of protein and amino acids. *Journal of Agricultural and Food Chemistry* **28** : 848 – 850.
- Said, M. Z., D. D. Culley, L. C. Standifer, E. A. Epps, R. W. Myers and S. A. Bonney. 1979. Effect of harvest rate, waste loading & stocking density on the yield of duckweeds. *Proceedings of the World Mariculture Society* **10** : 769 – 780.

JHA AND POKHAREL (BHATTARAI) – DUCKWEED : THE HIDDEN TREASURE

- Shahjahan, M., A. H. Khan, N. Akhtar, A. S. M. A. Rahman and F. Z. Majid. 1981. Use of aquatic weeds and algae as poultry feed. *Proceedings 2nd Annual Conference, Livestock*. Bangladesh Agriculture University, Mymensingh. pp. 272-278.
- Sheh – May, T., P. C. Boyce, T. M. Upson, D. Barabe, A. Bruneau, F. Forest and J. S. Parker. 2004. Intergeneric and intrafamilial phylogeny of subfamily Monosteroideae (Araceae) revealed by chloroplast sequences. *American Journal of Botany* **91** : 490 – 498.
- Shireman, J. V., D. E. Colle and R. W. Rottmann. 1977. Intensive culture of grass carp, *Ctenopharyngodon idella* in circular tanks. *Journal of Fish Biology* **11** : 267 – 272.
- Skillicorn, P., W. Spira and W. Journey. 1993. *Duckweed Aquaculture : A New Aquatic Farming System for Developing Countries*. The World Bank, Washington, D.C.
- Stapp, W. B. and M. K. Mitchell. 1995. *Field Manual for Global Low-cost Water Quality Monitoring*. Green Office, Michigan.
- Sutton, D. L. and W. H. Ornes. 1975. Phosphorus removal from static sewage effluent using duckweed. *Journal of Environmental Quality* **4** : 367 – 370.
- Taubaeu, T. T. and M. Abdiev. 1973. *Duckweeds of Water Reservoirs in Uzbekistan and their Use in Agriculture*. Fan, Taskent.
- Upadhyay, B. P., S. Jha, U. Koirala and B. Niroula. 2011. Inventory of ten aquatic habitats of Biratnagar township, Nepal. *Geobios* **38 (4)** : 283 – 288.
- Van der Does, J. and F. J. Klink. 1991. Excessive growth of *Lemnaceae* and *Azolla* in ditches observed by false colour teledetection. *Verhandlungen des Internationalen Vereins fur Limnologie* **24** : 2683 – 2688.
- Vroon, R. and B. Weller. 1995. *Treatment of Domestic Wastewater in a Combined UASB – reactor Duckweed Pond System*. Landbouwniversiteit, Wageningen.
- Wangermann, E. and E. Ashbey. 1950. Morphogenesis in *Lemna minor*. *Proceedings Linnean Society of London* **162** : 10 – 13.
- Wang, W. and J. Messing. 2012. Analysis of ADP – glucose pyrophosphorylase expression during turion formation induced by abscisic acid in *Spirodela polyrhiza* (greater duckweed). *BMC Plant Biology* **12** : 274 – 290.
- Wolff, P. and E. Landolt. 1994. Spread of *Lemna turionifera* (Lemnaceae), the red duckweed in Poland. *Fragmenta Floristica et Geobotanica* **39** : 439 – 451.
- Wyman, C. E. 2003. Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and powder. *Biotechnological Progress* **19 (2)** : 254 – 262.
- Zaher, M., N. N. Begum, M. E. Hoq and A. K. A. Bhuiyan. 1995. Suitability of duckweed, *Lemna minor* as an ingredient in the feed of tilapia, *Oreochromis niloticus*. *Bangladesh Journal of Zoology* **23** : 7 – 12.
- Zirschky, J. and S. C. Reed. 1988. The use of duckweed for wastewater treatment. *Journal of the Water Pollution Control Federation* **60** : 1254 – 1258.
- Zutshi, D. P. and K. K. Vass. 1973. Ecology of macrophytic vegetation of Kashmir lake. In C. R. Varshney and J. Rzoska (eds.) *Aquatic Weeds in S. E. Asia*. Junk, The Hague. pp. 141-146.

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SEASONAL VARIATIONS IN BIOMASS AND NUTRITIVE VALUE OF TWO DUCKWEED SPECIES AT BIRATNAGAR, NEPAL

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Key words: Lemna aequinoctialis, Spirodela polyrhiza, water quality

ABSTRACT

The tiny aquatic angiospermic plants "duckweeds" have a prominent value. seasonal variations in physico-chemical properties of water and biomass and nutritive value of two duckweed species (*L. aequinoctialis* and *S. polyrhiza*) occurring intermixed in a roadside pool at Biratnagar (latitude N 26°20'; longitude E 87°16'; altitude 72m, msl), Nepal was observed.

INTRODUCTION

In recent years, the tiny aquatic angiospermic plants "duckweeds" have become prominent because they provide high protein animal feed, organic fertilizer, bio-fuel; control mosquitoes; and, have great applicability in wastewater purification, toxicity testing, and in basic research and evolutionary model system [Jha & Pokharel (Bhattarai), 2015]. The present study deals with seasonal variations in physico-chemical properties of water (temperature, pH, total dissolved solids, nitrogen, phosphorus, potassium) and biomass and nutritive value (protein, phosphorus, potassium) of two duckweed species (*L. aequinoctialis* and *S. polyrhiza*) occurring intermixed in a roadside pool at Biratnagar

(latitude N 26°20'; longitude E 87°16'; altitude 72m, msl), Nepal.

The pool is 40 m long and 3.5 m wide with water depth in the centre 0.45 m in summer and 1.12 m in rainy season. It receives nutrients through domestic wastewater and agricultural run-off. The vegetation of the shore area (0 - 0.20 m water depth) was composed of *Alternanthera philoxeroides*, *Arundinella bengalensis*, *Colocasia esculenta*, *Cyperus pilosus*, *Eichhornia crassipes*, *Ludwigia adscendens*, *Mikania micrantha*, *Polygonum glabrum*, *Rumex dentatus* and *Sacciolepis indica*; whereas *L. aequinoctialis*, *Nitella mucronatus*, *Potamogeton crispus* and *S. polyrhiza* occupied deep water (> 0.20 m depth) zone

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of the pool. The base-layer soil (mud) had 49 % silt, 26 % sand, 25 % clay, 0.25 % nitrogen, 0.001 % phosphorus, 0.076 % potassium, 3.96 % organic carbon, and 15.84 : 1 C : N ratio.

Intermixed duckweeds were sampled using ten 10x10 cm quadrat in the first week of January (winter), May (summer) and September (rainy season), 2015. The duckweeds were separated species-wise and squeezed gently between blotting papers before weighing for fresh biomass. Thereafter, the duckweed samples were dried in oven at 800C to constant weight. The dried samples were analysed for protein, phosphorus and potassium using the methods of AOAC (1990). APHA's (1998) methods were used for

the analysis of the physico-chemical parameters of water samples collected simultaneously with the duckweed samplings.

A variety of environmental factors, such as water temperature, pH, nutrient concentration, crowding by overgrowth of the colony, competition from other plants for light and nutrients etc., control the growth and survivability of duckweeds (Hasan & Chakrabarti, 2009). Tables 1 and 2 indicate that increase in the N, P, K concentrations in water was associated with increase in biomass and nutritive value (protein, P, K) of both *L. aequinoctialis* and *S. polyrhiza*. Edwards *et al.* (1992) observed that pond water with less than 3 mg/l N and 0.3 mg/l P did not support normal growth of *L. perpusil-*

Table 1: Seasonal variations in physico-chemical properties of water (mean \pm SE; n = 3)

	January	May	September
Temperature ($^{\circ}$ C)	17.2 \pm 2.4	30.6 \pm 3.2	31.2 \pm 3.2
pH	6.2 \pm 1.4	6.4 \pm 1.4	6.2 \pm 1.4
Total dissolved solids (mg/l)	127.4 \pm 6.5	267.0 \pm 9.8	650.0 \pm 14.7
Nitrogen (mg/l)	8.2 \pm 1.6	4.4 \pm 1.2	3.3 \pm 1.0
Phosphorus (mg/l)	0.5 \pm 0.4	0.1 \pm 0.2	0.1 \pm 0.2
Potassium (mg/l)	19.7 \pm 2.5	6.4 \pm 1.4	4.3 \pm 1.2

Table 2: Seasonal variations in fresh biomass (mean \pm SE; n = 10) and nutritive value (mean \pm SE ; n = 3) of *L. aequinoctialis* (LA) and *S. polyrhiza* (S P)

	January		May		September	
	LA	SP	LA	SP	LA	SP
Biomass (g/m²)	89.8 \pm 3.0	823 \pm 9.1	30.6 \pm 1.7	397.2 \pm 6.3	25.2 \pm 1.6	453.5 \pm 6.7
Protein (%)	20.2 \pm 2.6	19.6 \pm 2.5	14.1 \pm 2.2	15.5 \pm 2.3	16.3 \pm 2.3	16.6 \pm 2.3
Phosphorus (%)	0.7 \pm 0.5	0.9 \pm 0.5	0.5 \pm 0.4	0.6 \pm 0.4	0.5 \pm 0.4	0.6 \pm 0.4
Potassium (%)	2.3 \pm 0.9	2.1 \pm 0.8	2.1 \pm 0.8	2.1 \pm 0.8	1.9 \pm 0.8	1.3 \pm 0.6

la and *S. polyrhiza*, whereas Luond (1980) reported growth reduction in duckweeds only when P concentration in water dropped below 0.017 mg/l.

The basic concept of a duckweed wastewater treatment system is to farm local duckweed on the wastewater requiring removal of dissolved salts. Landolt & Kandeler (1987) reported that of all aquatic plants, duckweeds have the greatest capacity in assimilating the macro-elements N, P, K, Ca, Na and Mg. There are thousands of derelict ponds polluted to eutrophication levels in Nepal alone that could be potentially cleansed of much of their pollutants through duckweed farming. Besides resurrecting the pond, the generated duckweed biomass can serve as high-protein animal feed and/ or fertilizer for crop production.

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REFERENCES

- AOAC. *Official Methods of Analysis*, 13th Edition. Association of Official Analytical Chemists, New York, 1990.
- APHA. *Standard Methods for Examination of Water and Wastewater*, 20th Edition. American Public Health Association, Washington D.C. , 1998.
- Edwards, P. , Hassan, M. S., Chao, C. H. and Pacharaprakiti, C. Cultivation of duckweeds in septage-loaded earthen ponds. *Bioresource Technology*, 1992, 40, 109 - 117.

Hasan, M. R. and Chakrabarti, R. *Use of Algae and Aquatic Macrophytes as Feed in Small-scale Aquaculture: A Review*. Food and Agriculture Organization of the United Nations, Rome, 2009.

Jha, S. and Pokharel (Bhattarai), B. Duckweed : the hidden treasure. *Nepalese Journal of Biosciences*, 2015, 5 (1), 1 - 24.

Landolt, E. and Kandeler, R. *The family of Lemnaceae - A Monographic Study :*

Phytochemistry, Physiology, Application and Bibliography, Vol. 4. Veroffentlichungen des Geobotanisches Institut der ETH, Zurich, 1987.

Luond, A. Effects of nitrogen and phosphorus upon the growth of some Lemnaceae. In : *Biosystematic Investigations in the Family of Duckweeds (Lemnaceae)*, (ed.) E. Landolt. Veroffentlichungen des Geobotanisches Institut der ETH, Zurich, 1980, pp. 118 - 141.



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GROWTH ON DIFFERENT MEDIA AND WATER CLEANSING EFFICIENCY OF *SPIRODELA POLYRHIZA* (L.) SCHLEIDEN

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Key words: Greater duckweed, growth, roadside pools, nutrient media

ABSTRACT

In-situ growth of *S. polyrhiza* exhibited senescence and rejuvenation cycles in all the selected habitats, and maximum frond biomass (53 g/m^2) occurred in April (water temperature 26° C). For frond culture, the best concentration of inorganic fertilizer in borewell water was N : P : K :: 10 mg : 2 mg : 2 mg/l, whereas 2 g/l sucrose was the best organic fertilizer. Further, the fronds displayed considerable nutrient removal efficiency from the wastewater.

INTRODUCTION

Spirodela polyrhiza (greater duckweed, family Lemnaceae) is a small, free-floating angiospermic plant of still and slow-moving freshwater bodies at Birtanagar (lat. N $26^\circ 20'$; long. E $87^\circ 16'$; altitude 72 m, msl), Nepal. The plant body is a few cells thick ovate to orbicular frond, dark-green above and purplish beneath, bearing a cluster of adventitious roots. The plant has gained prominence in recent years because of its utility as a high protein animal feed (Skillicorn *et al.*, 1993; Leng, 1999), agricultural fertilizer [Lot *et al.*, 1979; Pokharel (Bhattarai) & Jha, 2017], biofuel potential candidate (Cheng & Stomp, 2009), and its ability to remove minerals from domestic sewage and industrial efflu-

ents (Ozengin & Elmaci, 2007). In aforesaid context, monthly variations in *in-situ* growth; *ex-situ* growth on different concentrations of NPK fertilizer, and sugars (glucose, sucrose, xylose); and water cleansing efficiency of *S. polyrhiza* were investigated in the present study.

MATERIALS AND METHODS

(a) *In-situ* growth: Three roadside pools receiving domestic sewage all year round were selected for the study of monthly variations in number, morphology (frond size and root length), and biomass of *Spirodela* between December 2016 and May 2017, as a downpour in the last week of May 2017 washed away most of the fronds from the

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selected habitats, thus hindering further investigation. Among the selected pools, the first one was located in the vicinity of Biratnagar bus-park (habitat - 1); habitat 2 was situated adjacent to the Koshi - project complex along Biratnagar - Jogbani (Rani) road; and habitat 3 in the vicinity of National Homeopathy College along Balbhadra highway. Average water depth in middle of all the selected pools was about 1m in December and 0.3 m in May.

Fronds were sampled in the first week of each month from all the selected pools using 10 cm x 10 cm quadrat (fitted with a handle and sieve) in triplicate. These were separated from those of *Lemna aequinoctialis* before their number was counted. About 50 fronds of the test species selected randomly were measured for size (graphically) and root length. The fronds were dried in oven to a constant weight at 80° C for biomass determination.

(b) *Nutrient media*: Different concentrations of inorganic fertilizer (combination of urea, diammonium phosphate and muriate of potash in 5 : 1 : 1 ratio), and sugars (glucose, sucrose, xylose) were made in borewell water. Three litre solution of each strength in triplicate was kept in plastic bowls along with a control (borewell water only) under laboratory conditions, and 1 g fresh *Spirodela* fronds of uniform size were incubated in all the bowls. Borewell water was added into the bowls at regular intervals to compensate for the evaporative losses. One fortnight after the commencement of each experiment, the incubated fronds were harvested for measuring

increase/ decrease in their fresh weight. Growth experiments on different media were conducted as per the following schedule: February 24 to March 14, 2017 (inorganic fertilizer); April 8 - 22, 2017 (sucrose); April 23 to May 7, 2016 (glucose); May 10 -24, 2017 (xylose).

(c) *Water cleansing efficiency*: Water samples were collected from a ditch located in the main vegetable market (Gudari) of Biratnagar on August 31, 2017. The ditch was used by vendors for dumping spoiled vegetables. These water samples were analysed for pH and nutrients (nitrogen, phosphorus, potassium) before incubating *Spirodela* fronds on them. Thereafter, 3 l ditch water was placed in plastic bowls in triplicate, and 1 g fresh *Spirodela* fronds were incubated on them. The cultured fronds were harvested one month after commencement of the experiment for measurement of fresh weight, and water was analysed further for assessing the changes in nutrient concentration.

The physico-chemical properties (temperature, pH, nitrogen, phosphorus, potassium) of water samples were measured as per the methods of APHA (1990).

RESULTS AND DISCUSSION

The optimum range of some important physico - chemical factors of water for the growth of duckweeds is as follows; temperature 15 - 30° C, pH 6.5 - 8, nitrogen 7 - 12 mg/l, and phosphorus 4 - 8 mg/l (Hasan & Chakraborti, 2009). Thus nitrogen and phos-

phorus concentrations in all the selected pools for the present study were inadequate for proper growth of the test species (Table 1).

The frond size ranged between 29.19 (February) and 72.06 mm² (April) in habitat 1, between 27.37 (March) and 43.17 mm² (February) in habitat 2, and between 24.1

(December) and 41.07 mm² (February) in habitat 3 (Table 2). Ashbey & Wangermann (1949), and Wangermann & Ashbey (1950) have reported similar cycles of senescence and rejuvenation in the vegetative growth of *Lemna minor* associated with periodic reduction/ increase in size and number of the frond cells.

Table 1: Nutrient concentration in the selected pools (mean \pm SE)

	Habitat 1		Habitat 2		Habitat 3	
	Jan	May	Jan	May	Jan	May
Nitrogen (mg/l)	5.25 \pm 1.32	3.37 \pm 1.05	4.74 \pm 1.25	3.37 \pm 1.05	2.46 \pm 0.90	0.98 \pm 0.57
Phosphorus (mg/l)	0.11 \pm 0.17	0.07 \pm 0.15	0.12 \pm 0.20	0.05 \pm 0.12	0.19 \pm 0.24	0.12 \pm 0.20
Potassium (mg/l)	19.70 \pm 2.56	6.40 \pm 1.45	4.99 \pm 1.28	4.57 \pm 1.23	13.20 \pm 2.09	7.65 \pm 1.59

Maximum frond biomass per unit area was 53 g/m² in April (water temperature 26^oC) in habitat 1, 21.06 g/m² in May (water temperature 27^o C) in habitat 2, and 41.07 g/m² in February (water temperature 15^o C) in habitat 3 (Table 2). Khondker *et al.* (1993) have reported that water temperature above 25^o C is detrimental to the growth of *S. polyrhiza*, whereas Pokharel (Bhattarai) & Jha (2016) have observed healthy growth of the test species even at 31.2^o C water temperature in a nutrient-poor roadside pool at Biratnagar, Nepal.

Root length of the test species ranged between 0.58 (May) and 1.17 cm (March) in habitat 1, between 0.68 (December) and 1.79 cm (February) in habitat 2, and between 0.78

(April) and 2.48 cm (December) in habitat 3. Leng (1999) opined that roots either stabilize the frond on the water surface or assist the frond to obtain nutrients where these are in dilute concentrations. They tend to lengthen as mineral nutrients in water are exhausted.

The best concentration of inorganic fertilizer per l of borewell water was N 10 mg, P 2 mg and K 2 mg for the growth of *Spirodela*. At this concentration, frond fresh weight increased by 59% than control after a fortnight incubation (Fig. 1). Higher concentrations of inorganic fertilizer had detrimental effect on frond growth. According to Poschenrieder & Barcelo (2004), higher NPK concentration disturbs the plant water status which eventually results in osmotic stress and growth reduction.

Table 2: Monthly Variations in some morphological attributes of *Spirodela polyrhiza* in selected habitats (mean \pm SE)

	Dec	Jan	Feb	Mar	Apr	May
Habitat 1						
Water Temperature ($^{\circ}$ C)	19.2 \pm 2.5	18.1 \pm 2.4	18.5 \pm 2.5	23 \pm 2.8	26.7 \pm 3.0	28.4 \pm 3.1
Water Ph	6.2	6.4	6.2	6.2	6.4	6.2
FronD size (mm ²)	42.80 \pm 3.77	31.11 \pm 3.22	29.19 \pm 3.11	61.11 \pm 4.51	72.06 \pm 4.90	43.69 \pm 3.81
FronD number/ m ²	46200 \pm 124	50866 \pm 130	3700 \pm 35	13533 \pm 67	40533 \pm 166	28940 \pm 98
FronD weight (g/ m ²)	22.56 \pm 2.74	17.58 \pm 2.42	0.35 \pm 0.34	11.39 \pm 1.94	53.00 \pm 4.20	21.87 \pm 2.7
Root Length (cm)	0.80 \pm 0.51	0.89 \pm 0.54	0.75 \pm 0.5	1.17 \pm 0.62	1.09 \pm 0.60	0.58 \pm 0.43
Habitat 2						
Water Temperature ($^{\circ}$ C)	19.0 \pm 2.5	17.0 \pm 2.4	18.8 \pm 2.5	22.9 \pm 2.8	26.7 \pm 3.0	27.5 \pm 3.0
Water Ph	6.3	5.8	6.0	6.3	6.2	6.3
FronD size (mm ²)	28.85 \pm 3.10	30.65 \pm 3.19	43.17 \pm 3.79	27.37 \pm 3.02	31.42 \pm 3.23	28.42 \pm 3.07
FronD number/ m ²	21633 \pm 84	39438 \pm 114	15726 \pm 72	64067 \pm 146	20300 \pm 82	36167 \pm 109
FronD weight (g/ m ²)	12.25 \pm 2.02	12.96 \pm 2.07	5.03 \pm 1.29	9.35 \pm 1.76	9.00 \pm 1.73	21.06 \pm 2.64
Root Length (cm)	0.68 \pm 0.47	1.27 \pm 0.65	1.79 \pm 0.77	1.50 \pm 0.70	1.67 \pm 0.74	1.52 \pm 0.71
Habitat 3						
Water Temperature ($^{\circ}$ C)	17.4 \pm 2.4	17.2 \pm 2.4	16.5 \pm 2.3	20.7 \pm 2.6	27.0 \pm 3.0	27.7 \pm 3.0
Water Ph	6.2	5.8	6.2	6.2	6.3	6.2
FronD size (mm ²)	24.10 \pm 2.83	24.98 \pm 2.88	41.07 \pm 3.7	34.25 \pm 3.37	34.41 \pm 3.38	36.11 \pm 3.46
FronD number/ m ²	49700 \pm 128	59100 \pm 140	32800 \pm 104	18400 \pm 78	48700 \pm 127	38767 \pm 113
FronD weight (g/ m ²)	36.45 \pm 3.48	38.22 \pm 3.56	14.39 \pm 2.19	8.88 \pm 1.72	16.04 \pm 2.31	21.00 \pm 2.64
Root Length (cm)	2.48 \pm 0.90	1.66 \pm 0.74	1.87 \pm 0.78	1.73 \pm 0.75	0.78 \pm 0.50	0.93 \pm 0.55

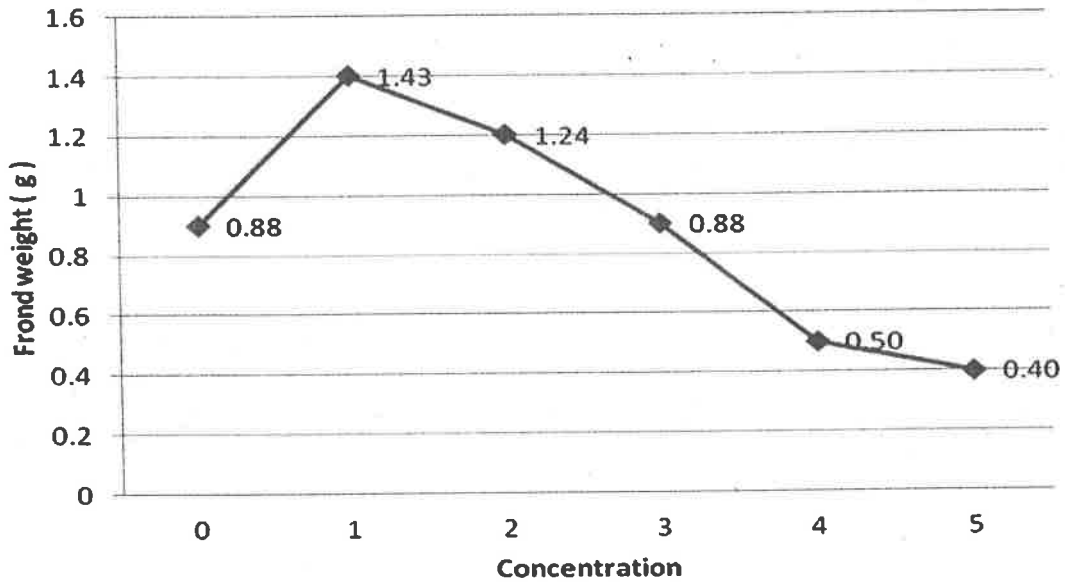


Figure 1: Increase/ decrease in fresh weight (1 g) of *Spirodela* fronds after incubation in different concentrations of NPK fertilizer per l of water for a fortnight; 0 = Control ; 1 = N : P : K :: 10 mg : 2 mg : 2 mg; 2 = N : P : K :: 20 mg : 4 mg : 4 mg; 3 = N : P : K :: 30 mg : 6 mg : 6 mg; 4 = N : P : K :: 40 mg : 8 mg : 8 mg; 5 = N : P : K :: 50 mg : 10 mg : 10 mg

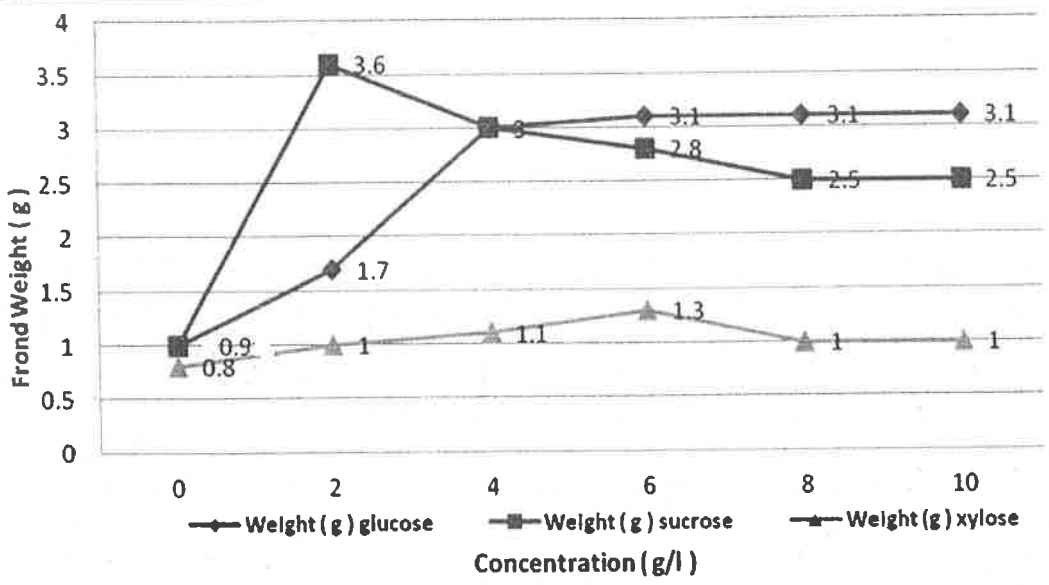


Figure 2: Increase/ decrease in fresh weight (1 g) of *Spirodela* fronds after incubation in different concentrations of glucose, sucrose, and xylose

Although appropriate concentration of glucose, sucrose, and xylose in water for the frond growth was 6 g/l, 2 g/l, and 6 g/l, respectively, the best growth was observed in 2 g/l sucrose concentration in the present study (Fig. 2). Leng (1999) stated that like other photosynthesizing organisms duckweeds grow with only requirements for minerals, utilizing solar energy to synthesize biomass; however, they have the capacity to utilize pre-formed organic materials particularly sugars and can grow without sunlight when provided with such energy substrates. This property of

Spirodela can be used for the treatment of wastewater from sugar industry.

Culture of *Spirodela* fronds for 30 days reduced the concentration of major nutrients in wastewater in the order : nitrogen (25%) > potassium (24%) > phosphorus (22%) (Table 3). Landolt & Kandeler (1987) stated that of all aquatic plants, duckweeds have the greatest capacity in assimilating major-elements N, P, K, Ca, Na and Mg but require longer retention time to reduce nutrient concentrations to specific discharge limits.

Table 3: Improvement in water quality induced by the culture of *Spirodela* (mean \pm SE)

	Initial water quality	Changes in quality 30 days after culture
pH	7.4	7.2
Nitrogen (mg/l)	6.9 \pm 1.5	5.2 \pm 1.3
Phosphorus (mg/l)	1.8 \pm 0.8	1.4 \pm 0.7
Potassium (mg/l)	11.5 \pm 2.0	8.7 \pm 1.7
<i>Spirodela</i> biomass (g)	1.0	3.5 \pm 1.1

In brief, occurrence of senescence and rejuvenation cycles in *in-situ* growth, appropriate concentration of inorganic as well as organic fertilizer in growth medium for the plant culture, and wastewater treatment efficiency of *S. polyrhiza* were the major findings of the present study that would be helpful in further investigations.

REFERENCES

- AOAC. *Official Methods of Analysis*, 13th Edition. Association of Official Analytical Chemists, Washinton DC., 1990.
- Ashbey, E. and Wangermann, A. Senescence and rejuvenation in *Lemna minor*. *Nature*, 1949, 164, 187.
- Cheng, J. J. and Stomp, A. M. Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean - Soil, Air, Water*, 2009, 37 (1), 17 - 26.

- Hasan, M. R. and Chakraborti, R. *Use of Algae and Aquatic Macrophytes as Feed in Small-scale Aquaculture : A review*. Food and Agriculture Organization of the United Nations, Rome, 2009.
- Khondker, M. , Islam, A. K. M. N. and Nahar, N. A preliminary study on the growth rate of *Spirodela polyrhiza*. *Dhaka University Journal of Biological Sciences*, 1993, 2, 197 - 200.
- Landolt, E. and Kandeler, R. *The family of Lemnaceae - A Monographic Study : Phytochemistry, Physiology, Application and Bibliography, Vol. 4*. Veröffentlichungen des Geobotanisches Institute der ETH, Stiftung Rubel, Zurich, 1987.
- Leng , R. A. *Duckweed : A Tiny Aquatic Plant with Enormous Potential for Agriculture and Environment*. Food and Agriculture Organization of the United Nations, Rome, 1999.
- Lot, A. , Novelo, A. and Quiroz, A. The chinampa : an agricultural system that utilizes aquatic plants. *Journal of Aquatic Plant Management*, 1979, 17, 74 - 75.
- Ozengin, N. and Elmaci, A. Performance of duckweed (*Lemna minor* L.) on different types of wastewater treatment. *Journal of Environmental Biology*, 2007, 28 (2), 307 - 314.
- Pokharel (Bhattarai), B. and Jha, S. Seasonal variations in biomass and nutritive value of two duckweed species at Biratnagar, Nepal. *Geobios*, 2016, 43, 4 - 7.
- Pokharel (Bhattarai), B. and Jha, S. Effect of duckweed manure on growth and yield of chilli and brinjal plants. *Geobios*, 2017, 44, 31 - 34.
- Poschenrieder, C. and Barcelo, J. Water relations in heavy metal stressed plants. In Prasad, M. N. V. (ed.) *Heavy Metal Stress in Plants from Biomolecules to Ecosystems*, 2nd Edition. Springer, Berlin, 2004, 249 - 270.
- Skillicorn, P. , Spira, W. and Journey, W. *Duckweed Aquaculture : A New Aquatic Farming System for Developing Countries*. The World Bank, Washington DC. , 1993.
- Wangermann, E. and Ashbey, E. Morphogenesis in *Lemna minor*. *Proceedings Linnaean Society of London*, 1950, 162, 10 - 13.
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EFFECT OF DUCKWEED MANURE ON GROWTH AND YIELD OF CHILLI AND BRINJAL PLANTS

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Key words: *Capsicum annum* L., *Solanum melongena* L., duckweed manure

ABSTRACT

The height, branch number, leaf number, leaf area, and leaf weight (dry) were significantly highest in both chilli and brinjal plants treated with duckweed manure at 7-day intervals. The best frequency of manuring for fruit production was 7-day intervals for chilli and 14-day intervals for brinjal plants. The soil under manure treatment accumulated high concentration of P.

INTRODUCTION

Duckweeds are the smallest, fastest growing, and the simplest free-floating aquatic angiosperms belonging to the monocotyledonous family Lemnaceae. Two duckweed species commonly found at Biratnagar (latitude N 26°20'; longitude E 87°16'; altitude 72m, msl), are *Lemna aequinoctialis* Welwitsch and *Spirodela polyrhiza* (L.) Schleiden. Both the species occur together in eutrophic roadside pools, old ponds, rice fields and ditches. On account of their soft body, high moisture, and nitrogen content, duckweeds are highly suitable for land appli-

cation in organic farming [Landolt & Kandeler, 1987; Pokharel (Bhattarai) & Jha, 2016]. The present study was undertaken to investigate the effect of semi-liquid manure of duckweed mixture (*L. aequinoctialis* and *S. polyrhiza*) on growth and yield (fruit production) of chilli and brinjal plants, and on soil properties.

MATERIALS AND METHODS

Forty earthen pots of 25 cm diameter filled with garden soil were employed for each test species. Seedlings of the test species were obtained from a local farmer and planted at the rate of one seedling per pot on

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December 5, 2015. The duckweed mixture, collected from a roadside pool through nylon net, was squeezed and kept in three 15 l airtight plastic containers for two months at room conditions, on October 7, 2015, for the preparation of manure. For manure treatment, the test species were segregated into four sets of 10 pots each as follows: (i) Control (C), to receive no manure, (ii) 7-D, to receive 500 ml manure at 7-day intervals, (iii) 14-D, to receive 500 ml manure at 14-day intervals, (iv) 21-D, to receive 500 ml manure at 21-day intervals. The pots for 7-D, 14-D and 21-D treatments received first application of 500 ml duckweed manure on December 7, 2015, and thereafter manuring was done on specified dates.

All the pots received 2 l water at every 4-day intervals from the date of seedling planting, and weeds and pests were controlled till

the harvest of the test species. Fruit picking was carried out on April 11, 2016 (when over 80 % fruits had reached maturity) and again on April 18, 2016. The plants were harvested between April 18 - 20, 2016 for the measurements of height, branch number, leaf number, leaf area, and dry weights of leaf, stem, and root per plant.

Composite soil samples from control and manure treated pots were analysed for pH by glass-electrode pH-meter; organic matter by Walkley and Black's method; and N, P, K concentrations by AOAC (1990)'s methods at the end of the experiments.

Statistical analysis of data was done using analysis of variance (ANOVA) procedure and means were compared using Duncan's Multiple Range Tests (DMRT).

Table 1: Effect of duckweed manure on some growth parameters of chilli plants*

	C	7 - D	14 - D	21 - D
Plant height (cm)	26.81 a	32.37 b	27.62 a	26.09 a
Branch number	18 a	36 b	28 c	32 b
Leaf number	151 a	343 b	223 c	198 c
Leaf area (cm ²)	699.89 a	1530.90 b	1497.88 b	924.66 c
Leaf weight (g)	2.15 a	4.38 b	3.07 c	4.15 b
Stem weight (g)	2.80 a	5.35 b	4.07 c	2.92 a
Root weight (g)	2.78 a	2.67 a	2.38 a	3.13 b
Fruit number	17 a	44 b	32 c	29 c
Fruit weight (g)	1.10 a	1.18 a	1.22 a	1.20 a

*Means followed by the same letters are not significantly different using DMRT (P < 0.05)

RESULTS AND DISCUSSION

The Plant height, branch number, leaf number, leaf area, and dry weights of leaves and stems were significantly higher in manure-treated than those of untreated individuals of both chilli and brinjal plants (Tables 1 and 2). Root weight (dry) was not significantly different in manure-treated and untreated chilli plants, but significantly higher than control in brinjal plants treated with manure at 14-D and 21-D intervals. Fruit number per plant ranged between 17 (C) and 44 (7-D) in chilli, and 7 (C) and 15 (14-D) in brinjal plants although both the test species had non-significant variations in fresh weight per fruit under different frequencies of manure treatment and control. The present findings are in agreement with Lot *et al.* (1979) that the duckweed can be used as an organic fertilizer in agriculture via composting or by direct land application. Marschner (1986) opined that organic manures may increase soil fertility and thus the crop production potential possibly by changes in soil's

physico-chemical properties including structure, nutrient bioavailability, water holding capacity, cation exchange capacity, pH, microbial community and activity, etc.

Manure treatments had no significant effect on soil pH under cropping of both the test species (Table 3). The level of organic matter ranged between 3.84 (C) and 7.91 % (14-D) under chilli, and 4.78 % (21-D) and 6.43 % (7-D) under brinjal cultivation. Total N in soil samples was significantly higher in manured (7-D and 14-D) pots planted with chilli, but manure treatments had no significant effect on soil N concentration under brinjal cultivation indicating greater demand of the nutrient for growth by brinjal plants. Phosphorus concentration increased tremendously in manure treated pots planted with the test species. It might be attributed to the P content in the manure. Potassium concentration in soil was lower in manure treated soils than the control. It was probably due to removal of K from soil in larger quantity by the test species.

Table 2: Effect of duckweed manure on some growth parameters of brinjal plants*

	C	7 - D	14 - D	21 - D
Plant height (cm)	16.62 a	31.00 b	27.25 a	17.50 a
Branch number	8 a	14 b	14 b	9 a
Leaf number	38 a	82 b	59 c	45 a
Leaf area (cm ²)	1431.65 a	2981.81 b	1920.93 c	1636.36 a
Leaf weight (g)	5.74 a	10.04 b	6.94 a	5.39 a
Stem weight (g)	2.89 a	7.61 b	4.91 c	3.87 c
Root weight (g)	2.53 a	2.93 a	5.16 b	3.92 c
Fruit number	7 a	12 b	15 b	10 a
Fruit weight (g)	85 a	88 a	84 a	84 a

*Means followed by the same letters are not significantly different using DMRT (P < 0.05)

Table 3: Effect of duckweed manuring on chemical properties of soil*

	C	7 - D	14 - D	21 - D
A. Chilli sets				
Organic matter (%)	3.84 a	6.82 b	7.91 b	5.41 a
pH	6.6 a	6.2 a	6.0 a	6.0 a
N (%)	0.19 a	0.34 b	0.40 b	0.27 a
P (ppm)	4.42 a	118.41 b	74.58 c	112.10 b
K (ppm)	54.57 a	46.78 a	51.98 a	7.79 b
B. Brinjal sets				
Organic matter (%)	5.96 a	6.43 a	5.17 a	4.78 a
pH	6.2 a	6.6 a	6.3 a	6.2 a
N (%)	0.30 a	0.32 a	0.26 a	0.24 a
P (ppm)	4.92 a	65.79 b	119.57 c	118.57 c
K (ppm)	44.18 a	36.38 b	41.58 a	5.19 c

*Means followed by the same letters are not significantly different using DMRT (P < 0.05)

From the present study it may be concluded that duckweed manuring can enhance the yield of chilli and brinjal crops appreciably, and it can be a boon in ameliorating P-deficiency in soils.

REFERENCES

- AOAC. *Official Methods of Analysis*, 13th Edition. Association of Official Analytical Chemists, Washinton DC. , 1990.
- Landolt, E. and Kandeler, R. *The family of Lemnaceae - A Monographic Study : Phytochemistry, Physiology, Application and Bibliography*. Veroffentlichungen des Geobot-
- anischen Institutas der ETH, Stiftung Rubel, Zurich, 1987.
- Lot, A. , Novelo, A. and Quiroz, A. The Chinampa : An agricultural system that utilizes aquatic plants. *Journal of Aquatic Plant Management*, 1979, 17, 74 - 75.
- Marschner, H. *Mineral Nutrition of Higher Plants*. Academic Press, New York, 1986.
- Pokharel (Bhattarai), B. and Jha, S. Seasonal variations in biomass and nutritive value of two duckweed species at Biratnagar, Nepal. *Geobios*, 2016, 43, 4 - 7.

Use of the Greater Duckweed as feed for the Tilapia fish

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Abstract

The fresh weight gain of the Nile tilapia individuals reared on different feeds for 21 days occurred in the order: conventional feed (65%) > conventional feed and duckweed powder in equal proportion (37%) > duckweed powder (09%). The conventional feed and duckweed powder were insignificantly different from each other in protein and phosphorus, but significantly different ($P < 0.05$) in fat, total ash and calcium contents. The tilapia individuals reared on the mixture of the conventional feed and duckweed powder had significantly ($P < 0.05$) highest concentration of proteins (50.3%) than those reared on either conventional feed (45.34%) or duckweed powder (45.79%) alone.

Key words: Nutrients, *Oreochromis niloticus*, *Spirodela polyrhiza*

Introduction

The Nile tilapia is a cichlid fish native to Africa but nowadays cultured in Nepal's Terai region also. It is a highly nutritious fish, rich in omega 3 oil which is good for patients with heart ailments. The fish contains less bone than other common fish and has a good taste, making it popular among fish lovers. It can survive extreme conditions like low oxygen and abrupt changes in temperature (Kingdon, 1989).

In general, protein component in fish feeds is a very essential factor affecting growth performance of fish and feed cost. The easily available duckweeds (the smallest aquatic angiosperms/Lemnaceae) can solve the problem as they are rich in protein and dietary minerals and low in fiber. They do not produce toxic alkaloids and are palatable to a wide variety of domestic animals and fish (Landolt & Kandeler, 1987). Hence, the present study was undertaken to evaluate the comparative effects of conventional feed (CF) alone, mixture of CF and greater duckweed powder (GD) in 1:1 ratio, and GD alone on the growth and nutrient concentrations of the tilapia fish.

Materials and Methods

The tilapia fingerlings (length 9.97 cm, breadth 2.96 cm, weight 13.43 g) were obtained from the Fishery Section of the Nepal Agricultural Research Council (Tarahara Branch, Sunsari District) and transported to the Bigyan Bhawan (PG Campus, Biratnagar) in polythene bags partially filled with water and oxygen. They were released in a concrete tank for acclimatization for two days. Nine transparent glass aquaria (size 45 cm x 30 cm x 30 cm) filled up to two-third volume with borewell water and having aeration facilities were employed for rearing tilapia fingerlings (3 individuals per aquarium) in three sets of different diet. In the first set of three aquaria, the fish received only the CF; in the second set they received the mixture of CF and GD; whereas only GD was provided to the fishes in the third set of aquaria. Specified diet was provided daily at the rate of 20% of the body

weight of the fish following Kabir *et al.* (2009). Water in each aquarium was replaced with fresh borewell water at 2 days intervals. The experiment was conducted between February 3-24, 2017 during which the minimum and maximum atmospheric temperatures were 15.5 and 30°C, respectively.

Among the feeds, CF was prepared by mixing wheat flour, soybean flour and powdered local fishes in 2:2:1 ratio, whereas for GD, *Spirodela* biomass, collected from a pool adjacent to the PG Campus, Biratnagar, was washed, dried in oven at 80°C to a constant weight, and grinded to powder in an electric grinder. AOAC's (1990) methods were followed for all the biochemical analyses.

Results and Discussion

Tilapia reared solely on CF had the highest increment in weight (65%) in comparison to those reared on the mixture of CF and GD (37%) or GD (9%) alone (Table 1). The fish reared on CF alone were golden-white, while those reared on the mixture of CF and GD, and GD alone were white and blackish in appearance, respectively. Comparatively, CF had significantly higher ($P < 0.05$) concentration of fats although total ash and calcium were significantly ($P < 0.05$) higher in GD. However, proteins and phosphorus were slightly more in CF than the GD (Table 2).

Table 1. Morphological variations in Nile tilapia reared on different feeds for 21 days. (mean \pm SE; n = 3)

	Appearance	Length (cm)	Breadth (cm)	Weight (g)	Growth increment (%)
Conventional feed (CF)	Golden-white	11.23 \pm 1.93	3.73 \pm 1.11	22.19 \pm 2.72*	65 \pm 5*
Greater duckweed (GD)	Blackish	10.10 \pm 1.83	3.73 \pm 1.05	14.64 \pm 2.21	09 \pm 2
CF + GD	White	10.23 \pm 1.84	3.16 \pm 1.10	18.40 \pm 2.47*	37 \pm 4*

* Means significantly different ($P < 0.05$)

Table 2. Nutrients (%) in CF and GD (mean \pm SE; n = 3)

	Fats	Proteins	Total Ash	Calcium	Phosphorus
GD	1.56 \pm 0.72	19.21 \pm 2.52	22.99 \pm 2.76*	0.84 \pm 0.53*	0.27 \pm 0.30
CF	7.89 \pm 1.62*	22.98 \pm 2.76	4.27 \pm 1.19	0.13 \pm 0.20	0.40 \pm 0.36

* Means significantly different ($P < 0.05$)

Tilapia reared on the mixture of CF and GD contained more proteins (50.3%) than those reared on CF (45.34%) or GD (45.79%) alone. However, calcium and phosphorus concentrations were significantly ($P < 0.05$) highest in the fish reared on the CF in comparison to those reared on the mixture of CF and GD as well as GD alone (Table 3).

Table 3. Nutrient accumulation (%) in fish reared on different feeds (mean \pm SE; n = 3)

	Fats	Proteins	Ash	Calcium	Phosphorus
CF	10.09 \pm 1.83	45.34 \pm 3.88	20.25 \pm 2.60	0.56 \pm 0.43*	0.39 \pm 0.36*
CF + GD	11.32 \pm 1.94	50.30 \pm 4.09	19.24 \pm 2.53	0.42 \pm 0.37*	0.24 \pm 0.28
GD	11.44 \pm 1.95	45.79 \pm 3.91	18.24 \pm 2.46	0.21 \pm 0.26	0.31 \pm 0.32

* Means significantly different ($P < 0.05$)

It may be inferred from the present study that the freely available greater duckweed can partially substitute the costly commercial feed in tilapia culture which is in agreement with the previous findings on other duckweeds (Gaigher *et al.*, 1984; Kabir *et al.*, 2009).

Acknowledgements

Authors are thankful to the Nepal Agricultural Research Council (Tarahara Branch, Sunsari District) for the generosity in providing tilapia fingerlings for the present study. Thanks are also due to the Head, Department of Botany, Post Graduate Campus, Biratnagar for facilities.

References

- AOAC 1990. *Official methods of analysis*, (13th ed.). Association of Official Analytical Chemists, Washington DC.
- Gaigher, I.G., D. Porath & G. Granoth 1984. Evaluation of duckweed (*Lemna gibba*) as feed for tilapia (*Oreochromis niloticus* x *O. aureus*) in a recirculating unit. *Aquaculture* **41**: 235-244.
- Kabir, A.N., M.A. Hossain & M.S. Rahman 2009. Use of duckweed as feed for fishes in polyculture. *Journal of Agriculture and Rural Development* **7(1-2)**: 157-160.
- Kingdon, J. 1989. *Island Africa: The evolution of Africa's rare plants and animals*. Princeton University Press, New Jersey.
- Landolt, E. & R. Kandeler 1987. *The family of Lemnaceae- A monographic study: Phytochemistry, physiology, application and bibliography*, vol. 4. Veröffentlichungen des Geobotanisches Institut der ETH, Stiftung Ruebel, Zurich.

APPENDIX 2

PARTICIPATION IN

CONFERENCE, TRAININGS AND

PHOTOGRAPHS

(NATIONAL AND INTERNATIONAL)

APPENDIX 2: INTERNATIONAL & NATIONAL CONFERENCE PARTICIPATION

- 2.1 Participated in the **Ph D Research Methodology Training Workshop** at Kathmandu, Nepal on 25 - 30 Jestha 2071 (8 - 13 June 2014) organized by Central Department of Botany, IoST, Tribhuvan University in support of University Grants Commission (UGC), Bhaktapur.

- 2.2 Participated in **THE 7th NATIONAL CONFERENCE ON SCIENCE AND TECHNOLOGY (SCIENCE, TECHNOLOGY AND INNOVATION FOR NEPAL'S GRADUATION TO DEVELOPING COUNTRY STATUS)** organized by Nepal Academy of Science and Technology (NAST) at Kathmandu, Nepal in between March 29 - 31, 2016.

- 2.3 Participated in the **TRAINING ON COMMUNICATION SKILL DEVELOPMENT IN SCIENTIFIC RESEARCH** at Biratnagar, Nepal on 22 - 24 Asar 2073 (6 - 8 July 2016) organized by PG Campus, Biratnagar in support of UGC Nepal.

- 2.4 Participated in **the International Conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood** at Kathmandu, Nepal on January 10 - 12, 2017 organized by USAID-IPM-IL-Biodiversity Project.

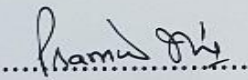
- 2.5 **Photographs:** National and International Conference Participation

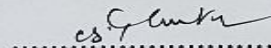


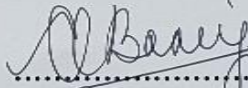
Research Methodology Training Workshop

CERTIFICATE

This certificate is awarded to **Bindu Pokhrel Bhattarai** for participating the PhD Research Methodology Training workshop organized by Central Department of Botany, IoST, Tribhuvan University and supported by University Grants Commission (UGC), Bhaktapur. This program was held from 25 - 30 Jestha 2071 (8 -13 June 2014).


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.....
Ms Chirika Shova Tamrakar
Dean, IoST
Tribhuvan University


.....
Dr Chitra Bdr Baniya
Coordinator
Central Department of Botany



Nepal Academy of Science and Technology

CERTIFICATE

Awarded to

BINDU POKHAREL BHATTARAI

for active participation/paper presentation/poster presentation

in

THE 7th NATIONAL CONFERENCE ON SCIENCE AND TECHNOLOGY

SCIENCE, TECHNOLOGY AND INNOVATION FOR NEPAL'S GRADUATION TO DEVELOPING COUNTRY STATUS

March 29-31, 2016

Kathmandu, Nepal

Mrs. Ramila Shrestha Raut
Chief, Promotion Division

Dr. Buddhi Ratna Khadge
Secretary

Prof. Dr. Jiba Raj Pokharel
Vice-Chancellor



Supported by UGC, Nepal



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Certificate

TRAINING ON COMMUNICATION SKILL DEVELOPMENT IN SCIENTIFIC RESEARCH

22-24 Asar 2073 (6-8 July 2016)

We are proud to present this certificate to

Bindu Pokharel (Bhattarai)

for active participation in the training

Dr. Manoj Kumar Raut
(Campus Chief)
Post Graduate Campus, Biratnagar

Prof. Dr. Sasinath Jha
(Head, Department of Botany)
Post Graduate Campus, Biratnagar

Prof. Dr. Damodar Thapa Chhetry
(Head, Department of Zoology)
Post Graduate Campus, Biratnagar

Prof. Dr. Shiva Kumar Rai
(Programme coordinator and Assistant Campus chief)
Post Graduate Campus, Biratnagar




Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms.

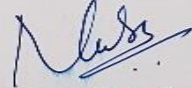
Bindu Pokharel

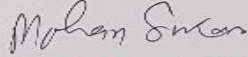
has presented paper/poster/attended
**the International Conference on Biodiversity, Climate Change Assessment
and Impacts on Livelihood**

at Kathmandu on January 10-12, 2017.

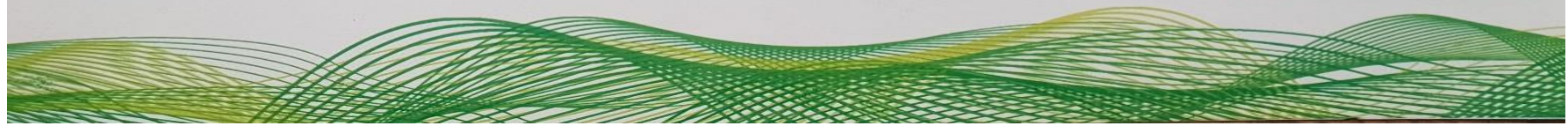

Dr. Bishwa Nath Oli
Secretary, Ministry of
Population and
Environment

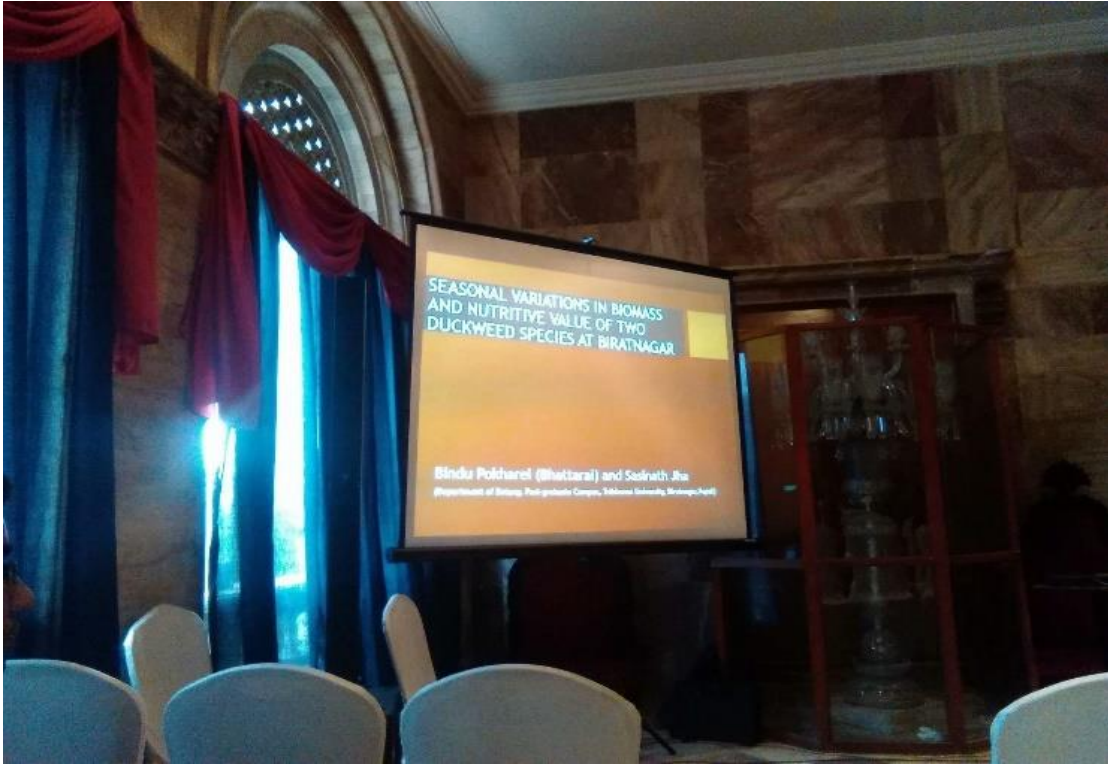

Dr. Nir Krakauer
Co-Chair, Conference
Organizing Committee
PI: USAID-IPM-IL-Biodiversity Project


Prof. Naba R Devkota
Director of Research
Agriculture and
Forestry University


Prof. Mohan Siwakoti
Secretary, Conference
Organizing Committee
Head, Central Dept. of Botany, TU


Prof. Pramod K Jha
Chair, Conference Organizing
Committee
Nepal Coordinator & Co-PI
USAID-IPM-IL-Biodiversity Project





Conference Participation: The 7th National Conference on Science And Technology, organized by Nepal Academy of Science and Technology in 2016



International Conference Participation: The International Conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood at Kathmandu, Nepal on in January, 2017