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Nutrient Recovery from Urine and Struvite Production Effluent Using Aquatic Plants in Nepal

Bachelor Thesis

by

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Fig. 1 (Front page): Experimental tanks with *Azolla* and duckweed in Siddhipur, Nepal

Abstract

The recovery of nutrients from urine for reuse in agriculture curbs the need to buy expensive commercial fertilizers and prevents eutrophication of water bodies through uncontrolled sewage discharge. However, direct field application of liquid urine is limited due to storage, transportation, and socio-cultural constraints. The precipitation of struvite ($MgNH_4PO_4$-$6H_2O$) is an option to trap almost all the phosphorus (P) from urine as a solid, storable and easily applicable fertilizer. The objective of the study at hand was to assess aquaculture in the context of Nepal as a possibility to recover the remaining nutrients from struvite production effluent– mainly nitrogen (N) and potassium (K) – or from urine itself, thereby producing protein-rich plant biomass that can be used as animal feed or green manure.

Based on a literature review, plant requirements and availability, the floating macrophytes *Azolla caroliniana* and *Spirodela polyrrhiza* were selected for outdoor experiments; the blue-green algae *Arthrospira platensis* had to be ruled out, largely because of low temperatures. The plants were grown in 35-L tanks with diluted urine and effluent. A control treatment with added diammonium phosphate (DAP) should resolve if a lack of P was the growth-limiting factor in effluent fertilized tanks. Over a 22-day period, photospectrometric analyses of growing medium samples determined removal of ammonium (NH_4-N), phosphate (PO_4-P), and K. The tanks were re-fertilized weekly to initial levels of 20mg·L^{-1} NH_4-N. At the end of the trial, biomass measurements assessed dry matter increase and total N content of *Spirodela*.

Azolla produced more biomass than *Spirodela* in all growing media (dry matter increase 327-452% vs. 204-277%), probably in part thanks to higher inoculation density. Better results for *Spirodela* might be achievable in shaded ponds and with higher initial coverage to reduce competition from algae, though *Spirodela*'s availability is limited in the winter. Tanks with added DAP showed lower biomass production but healthier plants and less algae. In *Azolla* grown on effluent, increasing signs of P deficiency became apparent (red coloration).

NH_4-N removal rates of 82-94% were recorded, being higher in *Spirodela* than *Azolla* tanks. The ratio of N assimilated by *Spirodela* was only marginal with ≤ 2.8% of total N removal. It must be assumed that most N was lost through other processes such as denitrification and volatilization of ammonia (NH_3). PO_4-P removal efficiencies from urine/effluent were higher in *Azolla* than *Spirodela* tanks (73/33% vs. 55/15 %). K analysis allowed no substantive interpretation due to low sample size.

The choice of methods, small number of replications as well as site-specific and lab-related challenges during measurements are likely to have influenced the outcome of the experiment and the obtained results. Nevertheless, it can be concluded that nutrient removal from urine with *Azolla* and *Spirodela* under the climatic conditions of early spring in Kathmandu is possible. Effluent as a growing medium can only be recommended for short-term treatment as P deficiency is expected to inhibit plant growth in the long run.

Further research needs include (1) the quantification of nutrients actually taken up by plants versus lost through other (abiotic) processes, (2) the investigation of year-round production feasibility, (3) an assessment of the need for and use of produced biomass as well as (4) the economic viability of nutrient recovery through *Azolla* and *Spirodela* in Nepal.

Zusammenfassung

Die Rückgewinnung von Nährstoffen aus Urin zur Wiederverwendung in der Landwirtschaft senkt den Bedarf an teuren chemischen Düngern und wirkt der Eutrophierung von Gewässern durch unkontrollierte Abwassereinleitung entgegen. Lagerungs- und Transportschwierigkeiten sowie soziokulturelle Faktoren schränken jedoch die direkte Verwendung von flüssigem Urin auf den Feldern ein. Die Fällung von Struvit ($MgNH_4PO_4$-$6H_2O$) ermöglicht es, einen Grossteil des Phosphors (P) aus Urin als festen, lagerfähigen und einfach auszubringenden Dünger einzufangen. Ziel der vorliegenden Arbeit war eine Eignungsabklärung von Aquakultur in Nepal zur Rückgewinnung der verbleibenden Nährstoffe – v.a. Stickstoff (N) und Kalium (K) – aus Struvitproduktionsabfluss (fortan „Effluent" genannt) und aus Urin selber für die Produktion von Pflanzenbiomasse als proteinreiches Tierfutter oder Gründüngung.

Ausgehend von Literaturrecherchen, Pflanzenbedürfnissen und –verfügbarkeit wurden die Schwimmpflanzen *Azolla caroliniana* und *Spirodela polyrrhiza* für Freilandexperimente gewählt; die blaugrüne Alge *Arthrospira platensis* musste u.a. wegen zu tiefer Temperaturen ausgeschlossen werden. Die Pflanzen wurden in 35-L Becken mit verdünntem Urin und Effluent einer Konzentration von 20mg·L^{-1} Ammonium (NH_4-N) herangezogen. Eine Kontrollbehandlung mit Effluent und zugegebenem Diammoniumphosphat (DAP) sollte zeigen, ob P der wachstumslimitierende Faktor in Effluent-gedüngten Becken war. Während des 22-tägigen Versuchs gaben photospektrometrische Analysen von Nährmediumproben Aufschluss über die Entfernung von NH_4-N, Phosphat (PO_4-P) und K. Am Ende des Versuchs wurden der Trockenmassezuwachs der Pflanzen sowie der totale N-Gehalt von *Spirodela* ermittelt.

Azolla produzierte in allen Nährmedien mehr Biomasse als *Spirodela* (Trockenmassezuwachs 327-452% vs. 204-277%). Bessere Resultate für *Spirodela* könnten wohl in beschatteten Teichen und mit höherem Anfangsdeckungsgrad zur Verminderung der Konkurrenz durch Algen erreicht werden; *Spirodela* ist im Winter allerdings beschränkt verfügbar. Becken mit zugegebenem DAP wiesen geringere Biomasseproduktion aber gesündere Pflanzen und weniger Algen auf. In Becken, die mit Effluent gedüngt waren, zeigte Azolla mehr und mehr Anzeichen von P-Mangel (rote Verfärbung).

Entfernungsraten von 82-94% NH_4-N wurden gemessen, wobei sie für *Spirodela* höher waren als für *Azolla*. Der durch *Spirodela* assimilierte N entsprach jedoch nur ≤ 2.8% der totalen N-Entfernung, so dass angenommen werden muss, dass N v.a. durch Denitrifikation und Ammoniakverflüchtigung verloren ging. PO_4-P Entfernung aus Urin/Effluent war höher in *Azolla*- als in *Spirodela*-Becken (73/33% vs. 55/15%). Der geringe Probenumfang erlaubte keine aussagekräftige Interpretation der K-Analysen.

Die Methodenwahl, die kleine Anzahl von Replikaten sowie ortsspezifische und labortechnische Schwierigkeiten bei den Messungen beeinflussten den Ausgang der Experimente wie auch die erhaltenen Resultate. Trotzdem kann zusammenfassend gesagt werden, dass Nährstoffentfernung aus Urin mit *Azolla* und *Spirodela* im Vorfrühling im Klima Kathmandus möglich ist. Effluent eignet sich nur kurzzeitig als Nährmedium, da längerfristig ein Wachstumsstopp durch P-Mangel zu erwarten ist.

Weiterer Forschungsbedarf besteht u.a. für (1) die Quantifizierung der Nährstoffaufnahme durch Pflanzen gegenüber dem Verlust durch abiotische Prozesse, (2) Abklärungen zu ganzjährigen Produktionsmöglichkeiten, (3) eine Ermittlung des Bedarfs an bzw. der Verwendung von produzierter Biomasse und (4) die Wirtschaftlichkeit der Nährstoffrückgewinnung durch *Azolla* und *Spirodela* in Nepal.

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Unless otherwise stated, all diagrams and tables were created and pictures taken by the author.

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Acronyms

Eawag	Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
ENPHO	Environment and Public Health Organization, Kathmandu
FAO	Food and Agriculture Organization of the United Nations
KU	Kathmandu University, Dhulikhel, Kavre, Nepal
NARC	Nepal Agricultural Research Council, Kathmandu
Sandec	The Department of Water and Sanitation in Developing Countries at Eawag
STUN	STruvite from Urine in Nepal, a project run by SANDEC in co-operation with UN-Habitat Nepal (→ chapter 2.3)
UN-Habitat	The United Nations Human Settlements Programme, collaborating in the Water for Asian Cities Programme and providing office space for STUN staff in Nepal
WHO	World Health Organization
ZHAW	Zurich University of Applied Sciences, Switzerland

Chemical Elements / Compounds and Other Abbreviations

N	nitrogen	Hg	mercury
P	phosphorus	Pb	lead
K	potassium	$(NH_4)_2HPO_4$	diammonium phosphate (DAP)
Na	sodium	$(NH_2)_2CO$	urea
Mg	magnesium	PO_4^{3-}	phosphate
Al	aluminum	NH_3	ammonia
S	sulfur	NH_4^+	ammonium
Ca	calcium	NO_3^-	nitrate
Cr	chromium	$MgNH_4PO_4$-$6H_2O$	magnesium ammonium phosphate, “struvite”
Mn	manganese	$MgSO_4$	magnesium sulfate
Fe	iron	CO_3^{2-}; HCO_3^-	carbonate; bicarbonate
Co	cobalt	NaCl	sodium chloride, salt
Ni	nickel	CH_4	methane
Cu	copper	HNO_3	nitric acid
Zn	zinc		
As	arsenic	EcoSan	ecological sanitation
Mo	molybdenum	PP	polypropylen
Cd	cadmium	DM	dry matter
Au	gold	CFU	colony-forming unit

1. Introduction

1.1 Context of the Research

Urine contains the greatest part of nutrients excreted by humans, which makes it a valuable resource, for example as fertilizer in agriculture (Kirchmann and Pettersson, 1995). In developing as well as developed nations, an increasing number of urine separation toilets are being installed, simultaneously tackling issues of sanitation and providing the opportunity to recycle nutrients locally. Besides curbing the need to buy expensive commercial fertilizers, extracting nutrients from urine can also reduce eutrophication of receiving water bodies through uncontrolled sewage discharge. However, the large volumes to be handled as well as seasonal and cultural constraints limit the applicability of liquid urine on fields. One option to address these problems is the recovery of nutrients in the form of user-friendly solid fertilizers such as struvite ($MgNH_4PO_4$-$6H_2O$).

In 2008, Eawag, the Swiss Federal Institute of Aquatic Science and Technology, and UN-Habitat Nepal, Water for Asian Cities Program, initiated the STUN project which was aimed at developing a low-cost process for precipitating struvite from urine at community scale in the village of Siddhipur in the outskirts of Kathmandu, Nepal (Gantenbein and Khadka, 2009; Etter, 2009). After the removal of struvite, however, a large volume of effluent remains. It contains hardly any phosphorus (P) anymore but still all of the initial potassium (K) and about 95% of the nitrogen (N) present in urine. If disposed of untreated, the effluent would therefore still pollute the environment and valuable nutrients would be lost.

A previous study (Kashekya, 2009) has already analyzed the suitability of the effluent for drip irrigation, but the issues of transportation and storage difficulties as well as some cultural objections to the use of liquid human fertilizer persist. It is thus necessary to investigate whether other processes allow the reuse of urine or struvite production effluent and therein contained nutrients directly on site. Aquatic plants such as algae and macrophytes are a promising possibility to recycle nutrients as they have been used for centuries to produce valuable biomass from domestic wastewaters (Iqbal, 1999; Leng, 1999; Lumpkin, 1983 and 1987).

1.2 Objectives and Approaches

The main objective of the Bachelor of Science thesis at hand is to investigate which aquatic plants are potentially suitable for nutrient recovery from both urine and struvite production effluent in Nepal and how they compare in terms of nutrient removal and biomass production. This is to be achieved through a literature review (Part I) and field experiments including laboratory analyses (Part II). The research is situated in the Kathmandu Valley, Nepal, and takes local circumstances into account.

Literature Review

A background chapter examines the potential of urine as a resource, aquaculture for nutrient recycling as well as the broader context of the project. It is followed by a theoretical assessment of three selected plant groups considered for nutrient recovery in the Nepalese environment: the blue-green algae spirulina (*Arthrospira platensis*), the family of *Azollaceae* and the subfamily of *Lemnoideae* (duckweed),

the latter two being floating macrophytes. These plants have been short-listed due to location factors, requirements and availability, great benefits of the produced biomass as well as previous and ongoing research.

The review is based on scientific papers and articles, subject-specific literature, direct communication with experts and, in the case of spirulina, a week-long training course on spirulina production by Antenna Green Trust in Madurai, Tamil Nadu (India).

Field Experiments

An experimental set-up is designed and field trials are conducted with two chosen plant species – the water fern *Azolla caroliniana* and the duckweed *Spirodela polyrrhiza* – using diluted urine and struvite production effluent as growing media. The field research consists of a preculture, a preliminary and a main experiment, taking place between January and March 2010 on a farm outside Siddhipur, Nepal.

The specific objectives are the measurement of nutrient removal efficiency from the growing media by the two plant species as well as the quantification of biomass production for further use in agriculture.

Laboratory analyses, carried out by the author at UN-Habitat in Nepal, in collaboration with and commissioned to ENPHO, Kathmandu University (KU), and Eawag Switzerland, determine nutrient removal from experimental tanks (ammonium (NH_4-N), phosphate (PO_4-P), and potassium (K)), dry weight and total N content of produced biomass as well as heavy metal and micronutrient content of utilized well water. In addition, temperature and pH are recorded three times a week.

A concluding chapter summarizes the main findings of the field experiments and includes suggestions for further research in the field of nutrient recovery from urine and struvite production effluent with aquatic plants in Nepal.

PART I: LITERATURE REVIEW

2. Background

2.1 Urine as a Resource

Urine is a valuable source of plant nutrients; the annual amounts excreted per person are considerable and exceed those excreted via feces (Table 1, based on data from Kirchmann and Pettersson, 1995).

Table 1: Annually excreted nutrients per person

	Urine	Feces
Nitrogen (N)	2.5 - 4.2 kg	0.5 - 0.7 kg
Phosphorus (P)	0.7 - 1.0 kg	0.3 - 0.5 kg
Potassium (K)	0.9 - 1.0 kg	0.1 - 0.2 kg

In times of rising prices of chemical fertilizers, depletion of non-renewable nutrient deposits such as phosphate rock, and pollution of water bodies due to uncontrolled discharge of sewage, the recovery of nutrients from urine seems an imperative and valid form of waste valorization.

Urine is generally sterile, though it may be contaminated with certain pathogenic organisms in the lower parts of the urinary tract (Schönning and Stenström, 2004). Feces, on the other hand, contain large amounts of microorganisms – including pathogenic ones – and pose the main risk regarding proliferation of gastro-intestinal vector diseases. In areas with unsanitary conditions, infection and transmission rates of such diseases are high, and it is not recommended that feces be used for direct field application (Kirchmann and Pettersson, 1995). Separate collection and treatment of urine and feces are therefore advisable for safe recycling of nutrients.

A new approach addressing this issue is ecological sanitation (EcoSan), aimed at closing the nutrient and water cycles by turning human excreta into sanitized fertilizers for agricultural use (UNEP/GPA et al., 2004). Source-separating systems (Fig. 2) with different storage tanks for urine and feces minimize cross-contamination and dilution with flush water and at the same time optimize the reuse potential.

Fig. 2: EcoSan toilet with separate collection of urine (front) and feces (back) (Water Aid, 2008)

Schönning and Stenström (2004) list the following advantages of urine separation:

- Less volume – the collection systems will fill up more slowly if urine is diverted
- Less smell – keeping urine and feces apart will reduce smell, making the use of the toilet and handling of excreta more convenient
- Lower risk of dispersal of pathogen-containing material through leaching to the groundwater and surrounding environment
- Safer and easier handling and use of excreta – drier feces are beneficial for pathogen reduction; the separate use of urine and fecal fractions is facilitated.

Feces may be used after appropriate storage times which depend on temperature, moisture, and pH (Schönning and Stenström, 2004). It can also be treated anaerobically for biogas production (Kirchmann

and Pettersson, 1995) or aerobically via composting, leading to stabilized and low-odor organic matter suitable for use in agriculture (Powers and Burns, 2004b). The involved processes shall not be further discussed in this thesis.

Urine can be sanitized through storage: At 20°C, inactivation of bacteria takes between 1 and 5 days; rotaviruses are reduced by 90% within 35 days (Schönning and Stenström, 2004). Lower temperatures require longer storage times; higher temperatures accelerate the inactivation of pathogens. Fecal cross-contamination through mismanagement or the inappropriate use of EcoSan toilets are to be avoided. During storage, urine should be kept in an air-tight container to prevent volatilization of ammonia (NH_3), and it should not be diluted because a concentrated milieu increases pathogen die-off. Stored urine may be directly applied to field crops, keeping a one-month interval between the last fertilization and harvesting. If edible parts grow above ground, urine should ideally be incorporated into the soil (ibid.).

Difficulties regarding the direct use of urine as liquid fertilizer in agriculture are the large volumes to be stored and transported and the seasonality of crop requirements vis-à-vis a constant supply. The latter is less of a problem in places with year-round production possibilities.

Compared to ammonium-nitrate (NH_4NO_3) fertilizers, urine application may lead to higher N losses because of volatilization and lower crop-uptake, whereas P from urine is at least as available as soluble phosphate fertilizers (Kirchmann and Pettersson, 1995). The loss of NH_3 can be reduced through drip fertigation, but the spontaneous formation of precipitates such as struvite may lead to clogging. Subjecting urine to prior struvite precipitation by adding magnesium (Mg) leaves an effluent that is better suited for drip fertigation because less prone to clogging (Kashekya, 2009).

Another reuse option is the addition of urine to compost heaps which may later be used as organic fertilizers e.g. for horticultural crops. Dosage of urine needs to be adjusted, though, because too much moisture results in anaerobic conditions and odorous compounds can be formed. Also, while P and K remain in the finished product, much of the N is volatilized during composting if the pile is not covered – with the consequence of negative effects on air quality (Powers and Burns, 2004b).

2.2 Reuse of Human Waste in Nepal

Unless otherwise stated, the information below is derived from a report by Water Aid Nepal (2008).

Traditionally, the ethnic Newar communities of the Kathmandu Valley used either raw or composted human waste for agricultural purposes. As discussed above, raw application of excreta presents a health risk, and the practice has been largely replaced since the introduction of chemical fertilizers. Composting used to play an important role: kitchen waste was dumped in a ditch in the backyard, and another pit on the ground floor under the staircase served to compost rice husk with ash and urine from children and elderly people who relieved themselves there out of convenience (Fig. 3).

Fig. 3: A traditional composting pit under the staircase (Gantenbein and Khadka, 2009)

In recent decades, traditional farming practices have declined in favor of industrialized methods such as the use of inorganic fertilizers and pesticides. In the process, nutrient cycles have been unwound, which has been further supported by modern forms of sanitation where excreta are flushed away – and often lead to water pollution – instead of being recycled on the spot (Mallapaty, 2010).

In view of these developments, EcoSan offers a compelling alternative by promoting both urgently needed village sanitation as well as the closure of disrupted nutrient loops at once. Since the introduction of the concept in Nepal in 2002/03, the number of EcoSan toilets in the country is estimated to have risen to over a thousand.

About 350 L of urine can be collected per adult person and year from EcoSan toilets (Gantenbein and Khadka, 2009). However, as mentioned above, a number of constraints limit the applicability of urine as liquid fertilizer:

In settlements of the Kathmandu Valley, the crop fields are often at a considerable distance from the farmhouse and the transportation as well as dilution and spreading of urine require significant additional efforts. Especially during the rainy season, people state to have surplus urine which they end up pouring out somewhere on their private land (ibid). Moreover, while most EcoSan toilet owners are aware of the beneficial fertilizing effects of dehydrated, compost-like feces to their soils, urine is often wrongly considered of not much value. An aversion to the handling of liquid urine as well as its strong odor may also act against widespread direct application. According to the results of a focus group discussion conducted in Siddhipur, about 50% of the farmers prefer a solid form of fertilizer such as struvite (Gantenbein and Khadka, 2009). Community members belonging to the ethnic group of Newars seem to be open to the use of different forms of urine in agriculture because of a long tradition of human waste recycling, but other Hindu casts (Brahmin, Chhetri) were found to be rather disinclined to use human waste in any form. The utilization of urine from households other than one's own has been particularly objected in the past (ibid.).

There are signs that attitudes are changing, though. The recent inauguration of a "urine bank" (a central urine collection system) in Siddhipur demonstrates that urine is increasingly recognized as a resource and its use is attracting more and more interest. The idea behind is that households with excess urine can have it picked up by a cyclist who transports it to a central storage tank, and farmers in need of urine to fertilize their soils can purchase it for a small fee on a rotational basis (Mallapaty, 2010).

A major advantage for farmers in Nepal is that using urine or its derivates makes them less dependent on market prices and the availability of commercial fertilizers. There is no chemical fertilizer industry in Nepal and those imported from India are subject to logistical problems as well as dubious quality due to a large informal sector (ibid.). Fossil fuels being a major component in the synthesis of mineral fertilizers, the price of the latter is highly influenced by the cost of crude oil (Leng, 1999). In 2007, for instance, prices for some fertilizers in Nepal multiplied by a factor of seven within a short period of time (Eawag, 2009). Another severe fertilizer shortage hit Nepal in the spring of 2010, right at the beginning of the rice planting season: the supply of chemical fertilizers in certain parts of the country could only meet about 10% of the demand (Ghimire, 2010) and farmers complained about black-marketing that forced them to pay double the price for supposedly subsidized fertilizers like urea, DAP and potash (Adhikari, 2010).

The crisis illustrated once more the vulnerability of farmers to volatile availability of agricultural inputs from the market. Since cattle are rare, composting and EcoSan toilets are the only sources of organic fertilizer (Gantenbein and Khadka, 2009).

From an environmental perspective, reusing nutrients from human urine may reduce the application of chemical fertilizers, thereby lowering the risk of soil degradation and eutrophication of receiving water bodies through leaching and runoff from excessively fertilized fields.

Furthermore, farmer Jiban Maharjan from Siddhipur has observed over years of urine application and comparative studies on his vegetable fields that plants fertilized with urine grow more vigorously, produce larger vegetables and achieve higher prices on the market than those treated with commercial fertilizers (Maharjan, 2010).

Fig. 4: Farmer Jiban Maharjan explaining the benefits of urine application

2.3 STUN Research Project

As indicated before, the STUN research project is run by Sandec, Eawag, in collaboration with UN-Habitat, and is aimed at the production of struvite from urine at community scale in Nepal. The project started in 2008 and will continue until the end of 2010.

Struvite ($MgNH_4PO_4$-$6H_2O$) is a dry, storable fertilizer that can be produced from urine through the addition of magnesium (Mg), e.g. in the form of magnesium sulfate ($MgSO_4$), pretreated magnesite rock ($MgCO_3$ turned into MgO), or bittern (a liquid by-product from salt production from sea water). The Mg ions bind with phosphate (PO_4^{3-}) and ammonium (NH_4^+) molecules, forming a solid precipitate that can be captured through filtration (Maurer et al., 2006). This mineral powder called struvite can then be turned into granules for easier application and used as slow-release P fertilizer. Compared to liquid urine, problems of storage, transportation, handling, and odor no longer exist, and even the use of struvite from mixed-source urine seems to be socially acceptable (Gantenbein and Khadka, 2009).

The technology of struvite precipitation has been extensively researched. In the United States, Canada, Europe, and Japan, large municipal sewage-handling facilities are already recovering phosphorus as struvite using full-scale systems (Ostara, 2010; Powers and Burns, 2004a). While such large-scale systems rely on high technology to recover P, the challenge in Nepal has been to design a community-scale low-cost struvite reactor that can be assembled from locally available materials and operated easily and efficiently with Mg inputs from a suitable source (Etter, 2009).

In a first phase of the project, a baseline study (Gantenbein and Khadka, 2009) was conducted to determine the current flow of phosphorus, i.e. urine production and chemical fertilizer use. A feasibility study further addressed the availability of resources, economic viability, and social acceptance of producing struvite in the community (Zurbrügg et al., 2008). The study concluded that struvite production in a peri-urban settlement of Kathmandu was possible, and the village of Siddhipur (Fig. 5) was chosen for further on-site research.

Fig. 5: Overview of Kathmandu and surrounding areas (Google Earth, 2010)

Siddhipur is a community of approximately 6000 inhabitants at about 8km southeast of Kathmandu. The population mainly consists of ethnic Newars and about 90% are involved in agriculture. Over the last years, 100 EcoSan toilets have been built within the community and are used with high degree of satisfaction (Manandhar et al., 2008). Besides the availability of excess urine from these toilets for struvite production, the village was selected because of the existence of a well-functioning user committee for water and sanitation issues and remarkable interest in and enthusiasm for struvite (Gantenbein and Khadka, 2009).

The second phase of the project then focused on the development, set-up and operation of a pilot struvite reactor in Siddhipur. The latter was designed for a capacity of 50 L of urine, consisting of a galvanized steel drum with a tapered bottom, central outlet and nylon cloth bag for filtration (see Fig. 21, chapter 4.2.2) (Etter, 2009). Urine is collected from different households by a cyclist hired for this purpose. The struvite reactor has turned out to function well with both $MgSO_4$ and bittern, achieving constant P recovery rates around 90% (ibid.), which corresponds with values from the literature (Powers and Burns, 2004a).

In the meantime an upscaled reactor with a capacity of 200 L has been installed at Kuleshwor Secondary School in Kathmandu, an excellent way to demonstrate the possibility of nutrient recovery from urine to a large number of students and teachers from whom the knowledge can spread further into the communities.

The precipitation of struvite mainly recovers PO_4^{3-} from urine; the supernatant – or effluent as it will henceforth be called – still contains a lot of valuable nutrients, namely N and K. Moreover, the process only removes a minor part of the initial volume of urine so that the quantities of effluent that require stor-

age and transportation to suitable cultures are still large. As indicated above, drip fertigation is one possibility to reuse effluent because of less clogging.

Aquaculture is another option to retrieve nutrients from either effluent or, sidestepping struvite production, directly from urine. The following chapter briefly reviews nutrient recovery through aquaculture, different possibilities of utilizing the produced biomass, and health issues that need to be considered.

2.4 Aquaculture for Nutrient Recovery

Most available literature is only on the use of human urine in combination with feces, possibly because urine separation is a fairly new approach.

Excreta-fed aquaculture has a long history in Asia. In China and Bangladesh (Fig. 6), for instance, human and livestock manures as well as contaminated surface waters have been used for hundreds of years to produce duckweed as feed for fingerlings or fish (ibid.; Powers and Burns, 2004b). On other continents, such practices are only marginal (WHO, 2006).

Fig. 6: Harvesting sewage-grown duckweed in Bangladesh (Iqbal, 1999)

The use of wastewater and excreta in aquaculture has a number of advantages:

- Better nutrition and improved household food security – products grown in aquaculture may make a substantial contribution to a balanced and quantitatively sufficient diet. The often protein-rich products grown in aquaculture may counter protein-energy malnutrition, the most lethal form of malnutrition especially in children, which leads to impaired growth, vulnerability to infection, and retarded cognitive development (WHO, 2006; Rai, 2007).
- The sale of products generates income and potential indirect health benefits (ibid.).
- Water reclamation – cleansing polluted water through biological mechanisms can make it potable and/or available for reuse, e.g. in agriculture where it is needed to produce enough food for a growing population (Leng, 1999).
- Wastewater treatment – aquacultures have the potential to be cheaper and more efficient than other treatment processes (Wilde and Benemann, 1993). The capacity of many plants to bioaccumulate heavy metals can even be used for remediating industrial wastewaters.
- Healthier environment – if nutrient-rich waters are channeled into aquacultures, the risk of negative impacts on ecosystems through uncontrolled discharge is reduced (Leng, 1999). The over-use of fertilizers has e.g. led to intolerable contamination of ground water supplies in some areas (ibid.).

Aquaculture requires considerable dilution of excreta so as to avoid exceedance of tolerance levels of the cultivated plants. A prerequisite for economically competitive aquaculture systems is a warm climate with a long growing season (Powers and Burns, 2004b).

2.4.1 Algae, Macrophytes, Polycultures

In developing as well as industrialized countries, biological treatment of wastewaters as an alternative to conventional processes is a field of intense interest (Rai, 2007).

The plants used include algae, e.g. *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Oscillatoria*, spirulina, and macrophytes, e.g. water hyacinth (*Eichhornia*), water lettuce (*Pistia*), duckweed (*Lemnoideae*) and *Azolla* (Bedell and Darnall, 1990; Leng, 1999*)*.

Polycultures that combine different species are run successfully in various parts of the world. They often comprise aquatic plants and fish that feed on them, either in the same or separate ponds. In China, systems with rice, *Azolla* and fish are known (Liu, 1987).

Besides removing nutrients like N and P, aquatic plants also produce biomass that can be harvested. In the case of algae, their removal from the treated effluent is a major cost and the large quantities to be handled (>200kg dry algal biomass per ha and day) require a convenient and economic solution (Laliberté et al., 1997). In general, the success of wastewater treatment through aquaculture depends on the usefulness (or easy disposal) of the produced biomass (Rai, 2007). Some of the options for subsequent use of plant biomass will be touched upon in the following chapter.

2.4.2 Reuse Options

Biofuel Production

Fuel production from agricultural crops is controversial, especially when the raw materials could be used for human consumption, e.g. in the case of corn where there is competition between the ethanol, food and feed industries.

For over a decade, duckweed (see chapter 3.3 for more information on the plant) has been investigated by Cheng and Stomp (2009a and b) as an attractive non-food alternative for fuel ethanol production. It produces 5-6 times more starch per acre than corn (45.8% of dry weight), can be processed and converted into ethanol in the same already existing facilities and results in an ethanol yield of 25.8% of the original dry biomass. At the same time, duckweed cultivation can serve to cleanse nutrient-rich wastewaters from municipalities or livestock production, esp. hog farms. The treatment ponds may be built on land unsuitable for conventional crops and the generated effluent water is clean enough for re-use, so that duckweed production is promising to become an environmentally friendly and economically viable technology.

Other researchers have studied the potential of biodiesel production from algae grown on wastewaters (e.g. Burton et al., 2009). Algae production uses little water, cultivation may even take place in areas where the ground water is salty, and biomass production is considerably larger than for higher developed plants (Läubli, 2008). Best suited for efficient algae production are regions with strong solar radiation. Compared to oilseed crops like canola and soybeans, algae contain up to ten times more lipids and their biomass yields as much as 30 times more oil than equivalent quantities of canola or sunflower seeds (ibid.). The recent rise in diesel prices is an increasing incentive, but in spite of published claims

that the technology is technically and economically feasible there are no operating large-scale algal biodiesel production processes so far (Burton et al., 2009).

Agricultural Use

The indirect use of excreta in agriculture, i.e. the production of animal feed or organic fertilizer, has the advantage of being acceptable in societies in which direct use is socially or culturally objected (WHO, 2006). Various taxonomic groups may be cultivated for animal feed: phytoplankton (e.g. *Scenedesmus*), zooplankton (e.g. *Daphnia*), or floating macrophytes with a high protein content. Likewise, when the intended use is biofertilization (green manure), algal suspensions as well as biomass from macrophytes can be applied, the latter either directly or via composting. The utilization of plant biomass in agriculture will be discussed in more detail in chapters 3.2.3 and 3.3.3, using the example of *Azolla* and duckweed.

Products for Human Consumption

Aquatic plants suitable for human consumption include water spinach, water chestnuts, water cress, water nuts, lotus, aquatic mint, and high-protein algae (spirulina). Edible animals that are directly or indirectly – via their feed – derived from excreta-fed aquacultural ponds include fish species (e.g. carps, *Tilapia*, catfish), mussels, prawns, crayfish, and fingerlings (Junge-Berberovic, 2001; WHO, 2006).

Raw Materials and Luxury Products

Apart from the use of plant biomass for renewable energy production (see above), the raw material of different species may be used for varied purposes, e.g. fibers for furniture and baskets (*Eichhornia*), cellulose for paper (*Typha*), or isolation material (also *Typha*).

Besides, a selection of optically attractive species can be marketed as luxury products such as ornamental plants (*Eichhornia* (Fig. 7), *Nuphar*) and fish (Koi – *Cyprinus carpio*) (Junge-Berberovic, 2001).

Fig. 7: *Eichhornia crassipes*, marketable as ornamental plant (Aquagarden, 2010)

2.4.3 Public Health Concerns

Unless otherwise referenced, all information in this chapter is based on WHO guidelines for the safe use of wastewater and excreta in aquaculture (WHO, 2006).

In wastewater or excreta-fed aquaculture, pathogenic organisms excreted by infected people can be transferred to others who either consume products grown in such waters or come in contact with the contaminated pond water, e.g. farmers running the aquaculture or local community members.

Pathogens that can lead to infections include bacteria (e.g. *E. coli*, *Salmonella spp*.), protozoa (e.g. *Giardia intestinalis*), helminths (e.g. tapeworms, *Fasciola*, *Fasciolopsis*, *Schistosoma spp.*), and viruses (various types, among them Hepatitis A and E). Many pathogens can survive in the environment long enough to pose a risk of transmission to humans. Factors influencing the die-off are temperature (pathogen survival is inversely related to temperature), moisture, UV radiation, absence of required

intermediate hosts, time, and the type of plants. According to Edwards et al. (1987), limited light as well as algal growth inhibit coliform removal in production ponds.

When plants are grown in an aquatic environment, any pathogen present in the water is likely to be present on the plants as well, especially on roots and other parts in contact with the water. Thermotolerant coliforms have been found to concentrate on the surface of duckweed, leading to levels 100 times higher than in the pond water. Metacercaria of trematode parasites may encyst on aquatic plants, remain infective for many months and even survive the winter in temperate climates. They are of particular concern because diseases associated with them have high morbidity. However, they require snails as intermediate hosts and can only multiply in ponds where such snails are present.

Fish accumulate bacteria, viruses, and protozoa in their digestive tract. Even in relatively clean water the microbial concentration in their guts is high. Studies have shown, though, that that the level of contamination is not higher for fish fed with duckweed from wastewater than for those fed with commercial feed.

The difficulty in assessing the presence of pathogens is the lack of a perfect indicator organism, especially for non-fecal pathogens that may also be excreted via urine. The concentrations of fecal indicator bacteria such as *E. coli* do often not correspond to concentrations of these organisms. Nevertheless, a microbial quality target of ≤ 10^4 *E. coli* per 100ml of pond water has been established to protect product consumers. For adequate protection from intestinal helminths, a threshold of 1 helminth egg per liter should not be exceeded.

Further preventive measures include the pre-treatment of wastewater or excreta in a series of stabilization ponds and the control of intermediate host populations. Sufficient hydraulic retention time (>20d) is reported to ensure pathogen removal and helminths die-off (Iqbal, 1999). For people working around wastewater ponds, the minimization of contact with contaminated water and personal hygiene help prevent transmission of diseases. Products for human consumption must be properly washed and inspected for helminth eggs. When processing fish, cross-contamination between gut contents and edible flesh should be avoided. Most importantly, thorough cooking of plants and fish prior to consumption is an effective means to reduce the risk of infection.

3. Species Considered for Nepal

3.1 Spirulina (*Arthrospira platensis*)

3.1.1 Characteristics

Taxonomy, Morphology, Reproduction

Spirulina is the common name given to the microscopic blue-green algae (cyanobacterium) *Arthrospira platensis* (see classification, Table 2). The algae consist of multi-cellular spiral-shaped filaments (trichomes) of up to 350µm length and a coil diameter of 44µm (Fig. 8). They are motile, showing cork-screw gliding movements (Antenna Green Trust, 2009).

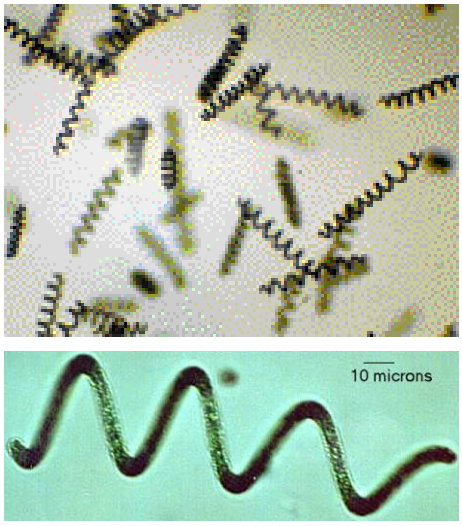


Fig. 8: *Arthrospira* (Kiani, 2007, top; Purdue University, 2010, bottom)

Table 2: Classification of *Arthrospira platensis* (Antenna Green Trust, 2009)

Division	Cyanophyta
Class	Cyanophyceae
Order	Nostocales
Family	Oscillatoriaceae
Genus	Arthrospira
Species	A. platensis (numerous varieties)

Spirulina has a typically prokaryotic cell organization without a nucleus. Its gas vacuoles regulate buoyancy and thus the amount of light received by the cells. High light intensity and photosynthesis increase turgor pressure, leading to the collapse of gas vesicles so that filaments temporarily sink to the bottom. During the day, the photosynthetic activity produces carbohydrates which are converted into protein at night (ibid.)

Reproduction takes place by simple cell division (binary fission) without any sexual or differentiation step (Vonshak, 1997). The trichomes are elongated through multiple intercalary cell divisions all along the filament; destruction (lysis) of an intercalary cell leads to fragmentation through breakage of trichomes (Antenna Green Trust, 2009). The daily growth rate is approximately 30% (Jourdan, 2006). Various researchers have reported algal production (dry matter) to lie between 8 and 12$g \cdot m^{-2} \cdot d^{-1}$ (cited in Vonshak, 1997). Productivity is directly correlated to CO_2 fixation and O_2 evolution – photosynthesis – which in turn are dependent on light and temperature. In cultures with high cell concentration, the specific growth rate increases linearly with the increase of the specific absorption rate of light energy. Heterotrophic growth of spirulina has been observed in cultures with glucose, but cells grown under such conditions have lower pigment content and show lower photosynthetic activity (ibid.).

Distribution

Spirulina naturally occurs on all continents, generally in alkaline, brackish and saline waters in tropical and semitropical regions between 35°S and 35°N. Based on size, shape, and climatic adaptation, many local varieties are distinguished. The variety native to India is called “Lonar” after a lake of the same name in the State of Maharashtra where it occurs (Antenna Green Trust, 2009). The natural occurrence of spirulina in Nepal has not yet been investigated.

Thanks to its filamentous form and the capacity to bioflocculate, spirulina is easier to harvest than other microalgae (Mohn, 1988). For many centuries, it has been collected from natural ponds, mainly in Latin America, Africa and South East Asia (Antenna Green Trust, 2009). Due to its beneficial nutritional properties it is now increasingly being cultivated in man-made tanks or even large-scale commercial production facilities consisting of open raceway ponds with paddle wheels (Vonshak, 1997). The following subchapters refer to active spirulina cultivation by man.

3.1.2 Requirements

Temperature, Rain and Wind

Temperature is one of the most important limiting factors in outdoor cultivation of spirulina, affecting all metabolic activities, nutrient availability and uptake, as well as the solubility of gases and other physical properties of the growing medium (Vonshak, 1997). For a healthy spirulina culture, specific strains which fit the local climatic conditions should be used (ibid.).

The optimal temperature for fastest spirulina growth is 35°C (Antenna Green Trust, 2009; Jourdan, 2006; Vonshak,1997). The extreme minimum temperature is 10°C (Vonshak, 1997) but growth virtually stops below 20°C (Antenna Green Trust, 2009). Temperatures below 20°C and above 45°C can cause irreversible damage and inhibit photosynthesis (ibid.).

Rain can be positive to compensate for evaporation but turns negative if the culture becomes too diluted or the tanks overflow (Jourdan, 2006). It is thus recommended to install a polythene sheet or greenhouse over the ponds (Antenna Green Trust, 2009). The latter can also improve temperature conditions in places with cool winters. Wind is beneficial for aerating and agitation of the culture but may cause pollution, e.g. through dust or particulates from roads and industries (ibid.; Jourdan, 2006).

Light

As a photoautotrophic organism, spirulina requires light for photosynthesis, but 10 hours per day are sufficient (Antenna Green Trust, 2009). Longer sunshine periods coupled with high temperatures and UV radiation lead to photolysis of cells, as does strong light with temperatures <15°C (Jourdan, 2006). Protein synthesis takes place in the dark.

Growth is proportional to available light, which in turn depends on surface radiation, culture depth, degree of turbulence and population density. Since spirulina is dark in color, a high concentration reduces light penetration. Sunlight generally passes through 17-18cm depth, hence the culture level should be maintained around 20cm to get optimal light and temperature conditions (Antenna Green Trust, 2009).

30% illumination saturates the photosynthetic capacity of spirulina but shading is not necessary unless evaporation, temperature of pH become too high. Full sunlight is preferred in the mornings in cool climates when quick heat-up is required (Jourdan, 2006). Exposure to high light intensities causes photoinhibition, i.e. a reduction of the rate of photosynthesis, maybe due to an accumulation of hydrogen peroxide (H_2O_2). High salinity and temperatures below the optimum make spirulina cultures more susceptible to photoinhibition (Vonshak, 1997).

Agitation

Agitation of the culture (Fig. 9) is essential for many reasons (Antenna Green Trust, 2009; Jourdan, 2006):

- Maintenance of a uniform culture temperature
- Even distribution of light (minimization of individual exposure to full sunlight, shorter periods of being shaded by other spirulina filaments) → increased productivity
- Homogenous dispersion of nutrients
- Increased dissolution of atmospheric CO_2
- Reduction of contaminants (e.g. insects, protozoa).

Fig. 9: Agitation of spirulina culture

Growing Medium / Nutrient Requirements

Spirulina requires a specific alkaline growing medium with the right combination of nutrients and salts. Any water added to the culture must be filtered and should originate from a borewell as opposed to an open source (e.g. river) because of the risk of contamination with other algae. Water from a supply system might be chlorinated, though, which is deadly to spirulina. Quantitative water requirements vary depending on regional rainfall patterns and evaporation rates. They are modest compared to conventional agriculture but water availability is necessary throughout the year (Antenna Green Trust, 2009).

Publications by Jourdan (2006) and Antenna Green Trust (2009) contain detailed data on the appropriate and convenient types and quantities of fertilizers to be used to obtain the desired composition and concentration of the growing medium. Only a few key nutrients shall be mentioned here.

Carbon (C) is the main nutrient and is needed at 3-4g·L^{-1} (Vonshak, 1997). While in nature, volcanic carbonate or atmospheric CO_2 supply C, the productivity of man-made spirulina cultures is usually increased with inorganic C fertilizers (e.g. sodium bicarbonate ($NaHCO_3$) or calcium carbonate ($CaCO_3$)), organic C sources such as biogas from cow dung, or additional CO_2 bubbled into the water. $NaHCO_3$ is popular because it also helps maintain required pH and salinity levels (Antenna Green Trust, 2009).

Otherwise, the usual major plant nutrients are required: N, P, K, S, Mg, Ca, Fe and a number of micronutrients, among others Cu, Zn, Mo, and Co. N must be provided on a daily basis, e.g. in the form of NH_3, NO_3^-, or urea ($(NH_2)_2CO$) (Jourdan, 2006). Concentration of urea in the growing medium must be kept below 60g·L^{-1} and NH_3 concentrations >30mg·L^{-1} may have toxic effects (ibid.; cf. chapter 3.1.4). Spirulina does not fix atmospheric N_2 (Antenna Green Trust, 2009).

P, Mg and Ca may cause imbalances in the growing medium through the formation and precipitation of $MgPO_4$ and $CaPO_4$ (Jourdan, 2006).

Since harvesting of spirulina biomass removes nutrients from the production ponds, they need to be replaced to maintain fertility of the growing medium. The fertilizers should be soluble or crystallized, not the “slow release” granulated type (ibid.).

pH

Proper pH is a crucial factor for the growth of spirulina and a defining parameter for nutrient uptake. It depends on photosynthetic activity and respiration; variations can occur due to excessive transpiration, a sudden change in the availability of nutrients (e.g. addition of carbonate salts), improper agitation or nutrient replacement after harvesting, improper maintenance of culture depth (Antenna Green Trust, 2009). Spirulina needs a high pH between 9.5 and 10.5 but not exceeding 11.3 (Jourdan, 2006). On the positive side, high pH conditions increase CO_2 absorption from the air (ibid.) and act as a barrier against contamination by other microalgae (Vonshak, 1997). However, they also promote the precipitation of $CaCO_3$ and sedimentation of algae. The decomposition of organic matter then increases bacteria populations, which eventually necessitates the complete replacement of the culture (Antenna Green Trust, 2009).

3.1.3 Possible Uses and Advantages of Biomass

Human Consumption

As indicated before, spirulina has a long tradition as human food on different continents. As early as the 16th century, indigenous people of Mexico were observed to harvest the biomass and sell dried cakes thereof in the market. Tribes of Chad and Niger were protected from drought-caused malnourishment thanks to the consumption of spirulina, and in parts of South-East Asia the algae has been used for over 1000 years in soups, spreads, and sauces (Antenna Green Trust, 2009).

Fig. 10: Spirulina production tanks at Antenna Green Trust, Madurai

Since the 1970s, massive spirulina production plants have been built to grow the valuable biomass as human food supplement. Nowadays spirulina is marketed and consumed all over the world (ibid.). It is mainly aimed at the health-food market but in a few developing countries production also takes place with simple techniques for the populations of rural areas (Dillon et al, 1995). For instance, Antenna Green Trust in Madurai, Tamil Nadu (India), produces spirulina candy for free distribution to school children to fight malnutrition.

Spirulina is justly praised for its nutritional qualities: When grown and harvested under appropriate conditions, it contains amazing 60-70% protein (Antenna Green Trust, 2009; Laliberté et al., 1997; Dillon et al., 1995), made up of 16 amino acids, 8 of them essential ones (Cohen, 1997). Fat content is low, as can be seen in Fig. 11 which shows the average composition of dried spirulina (based on values from Antenna NutriTech, 2010).

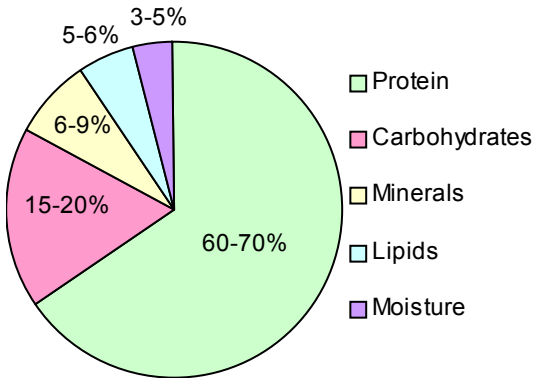


Fig. 11: Nutritional composition of spirulina

Besides the main organic nutrients, spirulina contains valuable minerals (Fe, Ca, Mg, P, K, various trace minerals), a large number of vitamins (e.g. vitamins B_1, B_2, B_6, B_{12}, D, E), pigments (among them phycocyanin; carotenoids: β-carotene (provitamin A), xanthophyll, myxoxanthophyll; chlorophyll α), and γ-linolenic acid (Antenna Green Trust, 2009; Cohen, 1997; Dillon et al., 1995). Since it has no cellulose cell-walls, spirulina is directly digestible (Antenna NutriTech, 2010; Dillon et al., 1995.).

The consumption of spirulina helps prevent nutritional problems such as protein energy malnutrition, anemia, and vitamin A deficiency. It also strengthens the immune system, lowers cholesterol levels, regulates blood sugar and pressure, improves gastrointestinal health and acts as a natural detoxicant (Antenna Green Trust, 2009).

Freshly harvested spirulina is most beneficial but it spoils after only a few hours at room temperature or a few days in the fridge. Drying (Fig. 12) and suitably packaging spirulina increases its shelf-life to about 5 years, but the process is expensive and makes the algae taste somewhat unpleasant (Jourdan, 2006).

Fig. 12: Spreading of spirulina for sun-drying

Animal Feed

Spirulina is also well accepted as nutritional supplement by various animals (Dillon et al, 1995). If fed to pigs, the production cost can be cut because drying of biomass is not necessary (Laliberté et al, 1997). The costs can be further reduced when wastewaters are used as nutrient source: Tanticharon et al. (1993) found that spirulina cultivated on secondary treated starch effluent from tapioca factories in Thailand, supplemented with HCO_3^- and fertilizers, was cheaper and at the same time a suitable feed for prawn and ornamental fish. Sun-dried spirulina produced on digested animal wastes or domestic raw sewage in India has also been incorporated into chicken rations at 5% without negative effects on health (Becker and Venkataraman, 1982; Saxena et al., 1983). Moreover, studies with chicken and quails showed that spirulina feed was a good yolk pigmenter thanks to its xanthophylls (Saxena et al., 1983; Cohen, 1997). Laliberté et al. (1997) point out that the safety of spirulina use as food and feed when grown on wastewater should be further investigated.

3.1.4 Constraints / Difficulties

Environmental / Seasonal / Ecological Constraints

Temperatures beyond the required range limit the possibility of spirulina cultivation, not least because spirulina is a continuous culture and not a seasonal crop that could be abandoned over the months with unfavorable climatic conditions (Antenna Green Trust, 2009). Ensuring adequate temperature throughout the year in a non-tropical climate is a major challenge. Addition of warm water (no more than 40°C to prevent damage) during the winter season may alleviate the problem, but since evaporation is minimal in cold weather, the amounts of water that can be added while maintaining a depth of 20cm are too small to substantially heat up the culture.

In December 2009, spirulina bucket cultures with inoculum from Antenna Green Trust in South India were started under the climatic conditions of Kathmandu (see temperature diagrams, chapter 5.4.1). They were kept indoors at night and outdoors during the day where they warmed up to around 25°C in the afternoon of sunny days. However, despite warm water inputs in the evenings, the temperature dropped to 13°C in the mornings. The use of electric water heaters to keep the temperature stable at the desired level (e.g. at 30°C) had to be ruled out due to frequent and long periods of load-shedding (up to 12 hours/day). In the long run or on a larger scale electric heating would neither be an environmentally friendly nor economically viable option in any case (Jourdan, 2006). The construction of a specially designed greenhouse could be attempted to trap maximum heat during the day, but maintaining the temperature above 20°C at night will hardly be possible in the winter in Kathmandu.

Limited availability of nutrients such as C, N, and P also restrains spirulina growth (Vonshak, 1997). Although urine has successfully been used as fertilizer in spirulina cultures, it cannot provide all the required nutrients: at least HCO_3^-, NaCl, and Fe have to be added (Jourdan, 2006). Struvite production effluent as a sole nutrient source is further expected to contain too little P in proportion to its N content.

At pH values above 9.25, NH_3 predominates over NH_4^+ and may be toxic, causing uncoupling of photosynthesis in algal cells. Although spirulina is less sensitive than other algae thanks to maintaining higher internal pH – some spirulina strains are even resistant to NH_3 toxicity –, considerable dilution of anaerobically digested waste is nevertheless needed to minimize negative effects (Laliberté et al., 1997).

There is a risk of contamination with green algae (e.g. *Chlorella*) through unfiltered water, especially when spirulina density is low (Antenna Green Trust, 2009). Excessive accumulation of organic matter and mud in outdoor cultivation can also inhibit the growth of spirulina and reduce its advantage over other algae (Jourdan, 2006). Bacterial contamination in connection with high pH has already been discussed above (chapter 3.1.2). Colonization by predators feeding on spirulina (e.g. larvae of mosquitoes, flies, amebae) reduces productivity; daily removal by netting and frequent agitation can curb the problem (Antenna Green Trust, 2009).

Economic / Cultural Constraints

Spirulina production is labor-intensive and time-consuming. Parameters of the culture medium such as pH, temperature, and viscosity need to be measured several times a day. Routine chemical analysis of nutrients is recommended to prevent depletion – e.g. through precipitation of P and Fe as well as biomass removal through harvesting – and the culture needs to be fertilized accordingly on a daily basis. Microscopic examination of spirulina filaments is essential to monitor the health state of the culture (Antenna Green Trust, 2009). To ensure agitation in intervals of 15-30 minutes, workers have to be employed. In bucket cultures, an aquarium-type air pump may suffice, but in larger tanks manual agitation is necessary as no optimal solution for automatic agitation has been found so far except for commercial spirulina factories. Harvesting (Fig. 13) requires filtration through two cloths of different pore

Fig. 13: Harvesting spirulina

size; washing with fresh water to get rid of the alkalinity and fertilizer residues; pressing to reduce the water content; drying and finally packaging or storage in opaque food-grade materials (ibid.).

Furthermore, a mother culture (i.e. a healthier spirulina culture grown in a shaded area and not harvested) needs to be maintained to serve as inoculum for new or weakened open air cultures, e.g. due to the invasion of microorganisms (bacteria, algae, fungal spores) (ibid.).

The costs associated with spirulina cultivation include capital investment, labor, water, nutrients and power for hygienizing the final product. Since only food-grade chemicals should be used as fertilizer, the cost of nutrients amounts to 15-25% of total production cost, C being one of the main components (Jourdan, 2006). The use of CO_2 from anaerobic digesters may lower the cost of inorganic C supply, but at the same time increases the risk of contamination with *Chlorella* species (Venkataraman et al., 1982).

Finally, if spirulina is grown on wastewater (including human and/or animal excrements), the product is expected to face major issues of acceptance as a human food supplement.

Health Issues

Spirulina readily absorbs and accumulates heavy metals (Jourdan, 2006). Both cultured and commercial spirulina have been found to contain about 9.5ppm mercury (Hg) so that chronic use may lead to Hg intake above prudent levels (Dillon et al,. 1995). When cultivated in pig wastes, the content of copper (Cu) – originally added to pigs' feed – may be too high and lead to cell mortality (Laliberté et al., 1997). If intended as food or feed, contamination of the biomass must be minimized, i.e. no industrial wastewaters with high amounts of heavy metals should be used (ibid.). Heavy metals in the algae may also be derived from the water and fertilizer used for cultivation (Dillon et al., 1995).

In addition, there is a possibility of contamination with toxin-producing cyanobacteria (e.g. *Anabaena*) that would make spirulina unsuitable as human nutritional supplements (WHO, 2006; Antenna Green Trust, 2009).

Most pathogenic bacteria cannot tolerate in the high pH range of a spirulina culture and helminths are not a problem because the required snail intermediate hosts do not survive in such conditions either (Antenna Green Trust, 2009). To eliminate remaining bacteria and viruses in harvested spirulina, the sun-dried flakes should be heated for an hour at 50-60°C. Also, sanitary conditions (e.g. protective clothing, personal hygiene) are essential when processing the biomass after harvesting (ibid.).

Fig. 14: Hygienic spirulina handling

3.1.5 Previous Research

Human and animal urine can provide N, PO_4^{3-}, SO_4^{2-}, Na, K, and Mg in "survival" situations and therefore replace some of the inorganic fertilizers (Jourdan, 2006). Likewise, bone meal can substitute Ca- and PO_4-salts (Becker and Venkataraman, 1982).

Animal wastes (especially pig wastes) have been found to be a good nutrient source for spirulina culture. Their use as fertilizer theoretically has the benefit of transforming potentially polluting nutrients into

valuable algal biomass and reducing the production costs of spirulina (Laliberté et al, 1997). However, pig wastes do not contain sufficient amounts of HCO_3^-, NO_3^-, K and NaCl for spirulina cultivation. NaCl needs may be satisfied by using seawater or natural salt water from brine springs (Olguin et al, 1994). Laliberté et al. (1997) state that though possible, it is not advantageous to grow spirulina in raw pig wastes for the following reasons:

- Economical impracticability because of the large (35fold) required dilution (cf. NH_3 toxicity above)
- Variability of composition of pig waste from one batch to another→ difficulty to optimize the system
- Prior aerobic / anaerobic fermentation of wastes is necessary to lower biological oxygen demand

They conclude that anaerobic digestion for methane (CH_4) production is a much more economically attractive option to treat animal wastes.

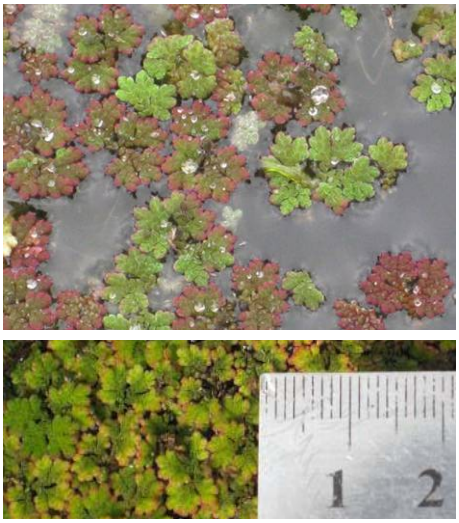
Fox (1987) has been working on combining different technologies to create integrated systems at village level in developing countries. His approach includes sanitation, biogas production, spirulina cultivation, composting, and fish culture. Human wastes are anaerobically digested and the produced CH_4 is used as cooking fuel. The remaining liquid effluent is filtered through a sand bed and treated by a solar sterilizer. Thereafter, it is used as fertilizer in spirulina cultures with the addition of $5g \cdot L^{-1}$ ocean salt and $4g \cdot L^{-1}$ $NaHCO_3$. After sun-drying and sterilization, spirulina is used as protein source for babies suffering from malnutrition and sold for fish cultures. Growing spirulina in an integrated system on animal wastes and seawater is expected to reduce production costs significantly compared to fresh water with NO_3^- and NaCl inputs, but the overall economic viability of integrated systems remains to be analyzed (Laliberté et al., 1997).

De la Noüe and Bassères (1989) found that when treating wastes biologically with cyanobacteria, one must decide between maximal nutrient removal rates and high biomass production as there is no obligatory correlation between the two parameters. The conditions necessary for high nutrient removal often lead to low biomass output rates and vice versa.

Removal rates of 87% N and 60% P in four days were reported from the first study on the potential of spirulina grown on secondary treated domestic wastewater which was supplemented with $NaHCO_3$ to adjust pH to 9.5 (Kosaric et al., 1974). However, reliable data on nutrient removal efficiencies is scarce and Laliberté et al. (1997) suggest that much of the removal may be due to abiotic rather than biotic factors. In fact, in a 75-L outdoor culture of another cyanobacteria (*Phormidium bohneri*), Proulx et al. (1994) found that a minimum of 62% of N removal was accounted for by NH_3 volatilization into the atmosphere. Regarding P, the environmental conditions required for spirulina favor abiotic PO_4^{3-} removal through precipitation (Laliberté et al., 1997; cf. nutrient requirements, chapter 3.1.2). Further research should thus attempt to quantify the proportion of N and P actually removed through biological assimilation by spirulina to avoid that the pollution is simply shifted from water to air (NH_3) or that valuable nutrients (PO_4^{3-}) precipitate unused (Laliberté et al., 1997).

3.2 Azolla (*Azollaceae*)

3.2.1 Characteristics



Taxonomy, Morphology, Reproduction

Azolla is an aquatic fern (see classification, Table 3) consisting of a floating, branching rhizome, with small, closely overlapping leaves and simple roots which hang down into the water (Hamdi, 1982) (Fig. 15). The plants can grow in multiple layers (Liu, 1987).

Table 3: Classification of *Azolla* (Lumpkin, 1983)

Division	Pteridophyta (Ferns)
Class	Filicopsida
Order	Salviniales
Family	Azollaceae
Genus	Azolla
Species	A.caroliniana, A.filiculoides, A.mexicana, A.microphylla, A.pinnata, A.nilotica, A.rubra

Fig. 15: *Azolla caroliniana*

Azolla lives in symbiosis with a blue-green algae (cyano-bacteria), *Anabaena azollae*, which is able to fix and assimilate atmospheric N_2 at a rate exceeding that of the legume / *Rhizobium* relationship, so that *Azolla* can survive on waters low in N but containing P. *Azolla*, in turn, provides C, a protective leaf cavity and a favorable environment for the algae. Part of the NH_4^+ fixed by *Anabaena* diffuses into the surrounding aquatic environment, providing an N source for flooded agricultural crops such as rice (Bhattarai et al., 2000; Kamalasanana Pillai (et al.), 2005 and 2008; Leng, 1999; Lumpkin and Plucknett, 1985).

Although *Azolla* has a sexual reproduction cycle with mega- and micro-sporangia, propagation generally occurs through vegetative reproduction (Hamdi, 1982). Multiplication is fast: *Azolla* is reported to double its weight within 2-5 days (Bhattarai et al., 2000; Lumpkin and Plucknett, 1985).

Distribution

The genus *Azolla*, native to Asia, Africa and the Americas, can now be found worldwide in water canals, ditches, ponds, and rice fields between latitudes of 55°N (Denmark, Alaska) and 55°S (Terra del Fuego), from near sea level to elevations up to 5000m (Lumpkin, 1983). There are two *Azolla* species in Nepal, the dominant one being *A. pinnata* which is present in all mid-hills and the Terai region (Thapa, 2009; Maskey and Bhattarai, 1977). However, it is difficult to maintain *A. pinnata* as a seed source to use as green manure in rice fields because of its susceptibility to low temperature so that cold-tolerant exotic species like *A. filiculoides* and *A. caroliniana* have been obtained from the International Rice Research Institute (Bhattarai et al., 2000). Nowadays *A. caroliniana* – which is originally from eastern South America but has been dispersed into other parts of the world (Lumpkin, 1983) – occurs naturally and abundantly in a number of ponds in and around Kathmandu (Thapa, 2009).

3.2.2 Requirements

Temperature and Humidity

Azolla is more cold tolerant than most other aquatic plants; *A. caroliniana* can survive in waters between about 0°C and 40°C (Wei et al., 1987). During the cool season there is little competition from other plants and damage from pests (Lumpkin, 1987), but water temperatures exceeding 40°C lead to death from heat stress (Lumpkin and Plucknett, 1985) as well as indirect detrimental effects due to insects, pathogenic fungi, and algae, which are further aggravated when coupled with high humidity (Lumpkin, 1987). Optimum temperature for most *Azolla* species is reported to lie between 20 and 25-35°C (Bhattarai, 2000; Lumpkin, 1987; Reddy, 1987).

Light

Azolla requires a photoperiod of 14-16 hours of daylight and 8-10 hours of dark (van Hove et al., 1987). There is a positive correlation between day length and growth rate (Lumpkin, 1987). Regarding exposure to sunlight, information in the literature is divergent: Whereas Kamalasanana Pillai (2008) states that direct sunlight is preferred and yields are lower in shady places, Lumpkin (1987) argues that 25-50% of full sunlight are sufficient and on the FAO feed resources website (FAO, 2010) shaded conditions are even recommended over full exposure to tropical sunlight.

Nutrient Requirements / Tolerance Values

Azolla requires all essential plant nutrients plus Mo or Co for N fixation (Lumpkin, 1987). It is sensitive to the application of combined N such as NH_4NO_3 or liquid N fertilizers, though, because they stimulate non-N-fixing competing species like weeds and algae (Hamdi, 1982; Kamalasanana Pillai, 2008; Shuying, 1987; Zhang, 1982).

P is the most common limiting element for *Azolla* growth; the P deficiency threshold value lies at 0.1 $mg \cdot L^{-1}$ in the water (Ali and Watanabe, 1987). P-stressed plants are smaller, pink to dark red with long, curled roots and have a low concentration of total N (Lumpkin, 1987). However, red coloration can also be due to intensive sunlight: direct exposure may turn the upper layer of plants red (Liu, 1987). Application of P fertilizer at a rate of 0.5-1.0$kg \cdot ha^{-1} \cdot week^{-1}$ P (=50-100$\mu g \cdot m^{-2} \cdot week^{-1}$) can overcome P deficiencies (Lumpkin and Plucknett, 1985). Adhikari et al. (1996) found that *Azolla*'s N content and N production were significantly higher with 4 split applications of P (at 5 day intervals).

Azolla is able to concentrate K from the water in which it grows (Liu, 1987); information regarding requirements and threshold values is scarce.

Salt content should be no more than 0.3%; above 1.3% *A. caroliniana* growth ceases (Lumpkin, 1987).

pH

For optimum growth pH should lie between 4.5 and 7, survival is possible in the range of 3.5 - 10 if all essential elements are available (Lumpkin, 1987). Low and high pH values may cause P to be tied up in insoluble compounds, making it unavailable for *Azolla* (Lumpkin and Plucknett, 1985). Kamalasanana Pillai (2008) therefore suggests in his instructions for backyard *Azolla* production that pH should never go below 5.5 or above 7.5.

3.2.3 Possible Uses and Advantages of Biomass

Green Manure

Azolla has been grown for centuries as N-fixing green manure for rice crops, especially in Vietnam and isolated areas of southeastern China. In other areas high temperatures and/or a lack of water often prevented cultivation of *Azolla* (Lumpkin, 1983 and 1987).

Either before transplanting rice seedlings or as an intercrop, *Azolla* is spread on the flooded fields and left to multiply. When it covers the surface, the water is drained and *Azolla* incorporated, slowly releasing its nutrients through decomposition and enriching the soil with 20-30kg·ha^{-1} N as well as considerable amounts of organic matter containing all essential plant nutrients (Bhattarai et al., 2000). Its humus helps maintain soil fertility, increases water holding capacity, promotes aeration, drainage, and aggregation essential for productive soils (Lumpkin and Plucknett, 1985; Chaudhary, 1994).

Azolla in paddy fields has equal to better efficiency than expensive chemical fertilizers and allows for gradual replacement of the latter (Gurung and Prasad, 2005; Kamalasanana Pillai, 2008; Liu, 1987). It is reported to increase rice grain yield by 17-25%, or even as much as 40% if incorporated a second time during the growth period. A dense cover of *Azolla* in rice paddies also increases the efficiency of water use by curbing evaporation and suppresses the growth of certain aquatic weeds by blocking out the sunlight and hindering their emergence. This reduces labor requirements for weeding, the most time-consuming activity in rice cultivation (Lumpkin and Plucknett, 1985). Moreover, research in Nepal has shown that *Azolla* significantly reduces NH_3 losses by buffering diurnal variations in floodwater chemistry and by direct N absorption (Tuladhar (and Vlek), 2002 and 2003).

Besides, the application of fresh or composted *Azolla* has favorable effects on field crops and vegetables (e.g. wheat, chilies, and potatoes) (Bhattarai and Maskey, 1977 and 1987; Hamdi, 1982).

Animal Feed

Azolla is an easily digestible and valuable source of animal fodder, containing only little lignin, carbohydrates and fat but between 13 and 35% of protein on a dry weight basis (Kamalasanana Pillai et al., 2005; Lumpkin and Plucknett, 1985). It provides almost all essential amino acids, vitamins (A, B_{12}), B-carotene, growth promoter intermediaries, and minerals like Ca, P, K, Fe, Co, Mg, and Zn. Its nutrient composition makes it a highly efficient and effective food for livestock which is reported to get accustomed to it quickly. Compared to protein-rich micro-fodder such as *Spirulina*, its production is low-cost and requires less skilled labor and care (Kamalasanana Pillai (et al.), 2005 and 2008).

Traditionally, *Azolla* has been used as animal feed for pigs, ducks and fish in Asia and parts of Africa (Lumpkin and Plucknett, 1985). When given to pigs, *Azolla* can completely replace commercial feed. For poultry, trials have shown that 20-25% of commercial feed such as soybean meal can be substituted with either dry or fresh *Azolla* without compromising the health of broilers (Kamalasanana Pillai, 2008; Paudel and Timsina, 2009). Positive effects of *Azolla* use include weight increase of broiler chicken, increased egg production of layers, and bigger, yellower yolks (Kamalasanana Pillai (et al.), 2005 and 2008). For dairy cattle, regular feed can be supplemented with 1.5-2kg·d^{-1} *Azolla*, which reportedly increases milk production by 10-20% and leads to higher milk fat content, improved health and

fertility of cows. Likewise, sheep and goats show increased weight and milk production as well as improved disease resistance and fertility when up to 30% concentrate is replaced with *Azolla* (ibid.).

Human Consumption

Although not widely reported, there is potential to use *Azolla* for direct consumption by man. Lumpkin and Plucknett (1985) write of a tasty dish of deep-fried *Azolla* mixed with batter in India; Hamdi (1982) mentions its use as a cooked leafy vegetable.

3.2.4 Constraints / Difficulties

Environmental / Seasonal Constraints

Azolla has several environmental constraints, e.g. intolerance of heat and high humidity as mentioned above (chapter 3.2.2). It can only survive for a few minutes on a dry surface under the tropical sun – sufficient water at a temperature below 40°C is compulsory for its survival (Lumpkin, 1987).

In addition, water availability is central to maintain nursery stocks with 1-10% of inoculation requirements of *Azolla*. Such nursery stocks must be maintained throughout the year when *Azolla* is not being cultivated in the fields because large-scale use of spores is not yet possible (ibid.).

Azolla may also have problems with continuous growth due to insect damage (Leng, 1999). Especially in the summer the plants may be attacked by larvae of moth and midge species as well as certain kinds of beetles and snails (Lumpkin and Plucknett, 1985). On the other hand, Wei et al. (1987) report *A. caroliniana* to have broad-spectrum stress-tolerance regarding insects, water algae, snails, and mildew.

Economic / Cultural Constraints

Apart from China and Vietnam, growing an aquatic crop as green manure is not a traditional agricultural practice, and since labor-intensive management and skills are necessary to ensure *Azolla*'s survival and propagation, it is not easily adopted (Lumpkin, 1983; Lumpkin and Plucknett, 1985; Sherchan and Karki, 2006). According to Khadka (2009) from the Nepal Agricultural Research Council (NARC), most Nepali farmers are not aware of the benefits of *Azolla* to soil fertility and crop production; many believe that *Azolla* is a weed and want to get rid of it. Even where the positive effects are known, few go to the trouble of draining the fields and incorporating *Azolla* – application of urea is easier and requires less labor (Khadka, 2009). The use of *Azolla* as fodder might also face difficulties of acceptance in places where this practice is new or the raising of typical *Azolla* foraging animals – pigs, poultry, fish – uncommon (Lumpkin and Plucknett, 1985).

Health Issues

In addition to the health risks mentioned in chapter 2.4.3, *Azolla* accumulates heavy metals such as Cr, Ni, Cu, Zn, Cd, Hg, and Pb from waters (Khosravi et al., 2005; Bennicelli et al., 2004), which inhibits growth and can have toxic effects on consumers of the plants. Where natural water bodies are used for cultivation, this does not normally pose a problem because the concentrations of metals are extremely low. However, plants grown on effluents from tanning or leachate from mining industries should not be used in any part of a food chain leading to human consumption (Leng, 1999).

3.2.5 Previous Research

A large number of scientific studies and projects have been concerned with *Azolla* cultivation as a means of recovering nutrients from different types of wastewater and/or producing biomass for further use. Those below are only a small selection of the existing literature:

DeBusk and Reddy (1987) conducted a number of studies to assess *A. caroliniana*'s growth characteristics, N-fixing capacity and potential to remove P and N from nutrient-enriched wastewater under greenhouse conditions. The results show that N_2 fixation rates are inversely related to NH_4^+ concentrations in the growing medium. When cultured in wastewater, *Azolla* absorbs a significant amount of N from the medium and does not solely rely on N_2 fixation as source of N (Reddy, 1987).

Shiomi and Kitoh (1987) studied the use of *Azolla* as decontaminant in sewage treatment and found that nutrient removal capacities of *Azolla* were lower for N but higher for P than those reported for other aquatic plants. Highest nutrient removal could be achieved when P levels were higher than N levels.

Costa et al. (1999, 2009) performed studies with *Azolla* on partially treated domestic wastewater, a suitable growing medium according to plant growth rates and productivity. Even high concentrations of NH_4^+ (34mg·L^{-1} N) did not seem to inhibit growth. Growth rates in diluted pig waste were lower, possibly because of increased presence of algae. *Azolla* was particularly efficient in the removal of P whose assimilation correlated significantly with the nutrient concentration in the growing medium.

Urine treatment by biological purification using *Azolla* and UV photocatalytic oxidation has been studied by Liu et al. (2008) in order to recover drinking water from urine in space stations. Trials showed that with *Azolla*, NH_4^+ in the urine solution could be reduced by >90% in five days.

The Vivekananda Kendra Natural Resource Development Project in Tamil Nadu, South India, has made positive experiences with growing *Azolla* on biogas residue (VK-NARDEP, 2006).

In excreta-fed duckweed ponds in Bangladesh (cf. chapter 3.3.5), *Azolla* has been observed to grow spontaneously and is successfully fed together with duckweed to a carp polyculture (Iqbal, 1999).

Rai (2007) examined the possibility of cultivating *A. pinnata* on sewage effluent to recycle wastewater and produce reusable biomass in the context of Northern India. He came to the conclusion that *A. pinnata* is suitable for cultivation on secondary-treated municipal wastewaters and that the produced biomass can safely be used as biofertilizer or green manure.

3.3 Duckweed (*Lemnoideae*)

3.3.1 Characteristics

Taxonomy, Morphology, Reproduction

Duckweed is the common name given to the simplest and smallest flowering, free floating aquatic plant belonging to the subfamily of *Lemnoideae* (see classification, Table 4). About 40 species are known worldwide, all of them with flattened, oval to round fronds (leaves) of max. 1cm across (Leng, 1999) (Fig. 16). Depending on the species and environmental conditions the plants develop root-like structures from a few mm up to 14cm for stabilization or improved nutrient uptake in diluted media.

Fig. 16: *Spirodela polyrrhiza*

Table 4: Classification of *Spirodela* (UniProt, 2010)

Division	Spermatophyta
Class	Magnoliophyta (Angiospermae)
Order	Liliopsida (Monocotyledons)
Family	Araceae (Lemnaceae)
Subfamily	Lemnoideae
Genus	Landoltia, Lemna, Spirodela, Wolffia, Wolffiella
Species	S.intermedia, S.polyrrhiza

Duckweed reproduces both vegetatively and sexually with sporadic and unpredictable flowering. Vegetative propagation occurs through clonal budding of new daughter fronds from two pockets on each side of the mature frond (Leng, 1999). Under ideal conditions in terms of water temperature, pH, incident light and nutrient concentrations, biomass can double within 24 hours, competing with the most productive terrestrial plants and even exceeding biomass accumulation of field crops such as corn (Cheng and Stomp, 2009a; Leng, 1999; Rodriguez and Preston, 1996).

Distribution

Species of the *Lemnoideae* subfamily can be found world wide, but the greatest diversity appears in subtropical and tropical areas. Their habitat comprises still or slowly moving fresh or polluted waters of only a few mm to 3m depth. In particular nutrient-rich and sheltered small ponds, ditches and swamps, e.g. down-stream from sewage works, often contain duckweed (Leng, 1999).

In Nepal, the occurrence of two species, *Lemna perpusilla* and *Spirodela polyrrhiza*, has been verified by the Herbarium of the Botanical Gardens in Godavari (Thapa, 2009). The plants can be found on their own or together with *Azollaceae* in ponds and irrigation canals (Fig. 17).

Fig. 17: Irrigation canal in Siddhipur with *A. caroliniana* and *S. polyrrhiza*

3.3.2 Requirements

Growth rates and nutrient content of duckweed are affected by temperature, light, possibly day length, the concentration, rate of replenishment and balance of minerals in the growing medium as well as water pH and initial biomass. If grown on very dilute media the main factors determining duckweed growth are incidence of sunlight, water and air temperature (Leng, 1999).

Temperature and Wind

Duckweed can grow between 6 and 33°C water temperature, with a positive correlation of growth rates and increasing water temperature up to around 30°C (Leng, 1999). It is therefore less sensitive to cool temperatures than other macrophytes (Iqbal, 1999), but extreme (low and high) temperatures adversely affect its growth (Edwards, 2010). Optimal temperature lies between 20 and 30°C, which should be considered when designing duckweed ponds because in shallow waters the temperature can quickly leave this range (Leng, 1999).

Studies in temperate and cold climates such as Central Europe have shown that under natural conditions duckweed growth is significantly reduced or completely stopped during winter months (Lüönd, 1983, Leng, 1999). When the temperature drops too low, duckweed can persist until favorable conditions return by producing starch filled structures or turin which are denser than the fronds so that the plants sink to the bottom (Leng, 1999).

Duckweed is sensitive to wind and is therefore not suitable to be grown in windy regions (Iqbal, 1999).

Light

High light intensity and direct exposure to sunlight can impede duckweed growth; shading is preferred (Edwards, 2010; Leng, 1999).

Nutrient Requirements / Tolerance Values

According to Leng (1999) only few studies have analyzed the best balance and concentration of nutrients for maximum growth of duckweed which also appears to depend on the initial composition of the plants. The literature presents contradictory values regarding optimum N concentration in the water, some authors including only NH_4-N, others giving no specifications concerning the chemical form of N. Whereas some suggest maximum concentrations of 20mg·L^{-1} NH_4-N (Caicedo et al., 2000; Rodriguez and Preston, 1996), the range is extended to 20-60mg·L^{-1} N by others (Leng, 1999, Nhapi, 2004), with optimum levels stated to lie between 40 and 60mg·L^{-1} N (Iqbal, 1999). There is agreement, though, that levels above 50-60mg·L^{-1} N coupled with a pH above 8 have a toxic effect on duckweed due to high levels of free NH_3 in the water (Caicedo et al., 2000; Iqbal, 1999; Leng, 1999; Nhapi, 2004).

If N- and P-levels are below 4mg·L^{-1}, duckweed cannot significantly reduce nutrients (Rejmankova, 1982). There are indications of symbiotic associations of N-fixing cyanobacteria with duckweed (genus *Lemna*) but their contribution to nutrient supply is considered insignificant compared to *Azolla*: on waters with low N, *Lemna* growth is slow and the product is low in protein (Leng, 1999). Since availability of NH_4^+ and ortho-PO_4^{3-} are limiting factors of fast duckweed growth, pretreatment of sewage is needed to release organically bound N and P if duckweed is used for further purification (Alaerts et al., 1996).

As for *Azolla*, P is the major factor limiting the occurrence of duckweed (Iqbal, 1999; Lüönd, 1983). In previous duckweed studies, P levels in water varied from 1.2-6.1mg·L^{-1} P (Leng, 1999).

Duckweed can efficiently remove K from polluted waters, but it is only needed in low concentrations to sustain growth, as long as other mineral requirements are met. In general K is sufficiently available from decaying plant material (ibid.).

Sulfur (S) is another essential nutrient, needed for the synthesis of S-amino acids in duckweed. Since it leaches easily from soil into water bodies it is not usually limiting in natural ponds but may become a limiting factor when NH_4-N content is in the optimum range and duckweed growth is vigorous (ibid.).

The non-governmental organization PRISM who has been running duckweed-fish-polycultures in Bangladesh for over 20 years noticed that the application of crude sea salt could provide sufficient trace minerals in production ponds otherwise fertilized by human excrements and inorganic fertilizers (Iqbal, 1999; Leng, 1999).

pH

Duckweed can survive at a pH between 5 and 9 but grows best in the range of 6.5-7.5. As mentioned above, high pH values lead to NH_3 in solution which can be toxic and also lost through volatilization (Iqbal, 1999; Leng, 1999).

3.3.3 Possible Uses and Advantages of Biomass

Animal Feed

Duckweed generally produces high annual yields per ha, is easy to harvest from the water surface (Iqbal, 1999), is palatable and has high digestibility for monogastric animals (Rodriguez and Preston, 1996). Its nutritional value is comparable to that of soybeans (Seidl et al., 2004). When duckweed is grown in nutrient-rich waters, its protein content (dry matter) almost doubles from 20-22% to 35-40% (Rodriguez and Preston, 1996). It also contains minerals, vitamins (A_1, B_1, B_2, B_6, C, E, PP), and pigments (xanthophyll, carotene), the latter deepening the yolk color of chicken eggs (Iqbal, 1999).

The most widespread application of duckweed is its use as fish feed, e.g. to Indian and Chinese carp species as well as tilapia, which often happens in polyculture systems (Iqbal, 1999). Because of its low carbohydrate and fat content, duckweed should be supplemented with conventional feed such as oil cake and wheat bran (ibid.). A study in the subtropical Terai region of Nepal has shown that duckweed is suitable as feed for Nile tilapia and common carp raised in polycultures over the summer months, and that it could potentially promote the productivity of aquaculture in Nepal (Shrestha and Bhujel, 1999).

Fig. 18: Duckweed as protein supplement for chicks (Rodriguez and Preston, 1996)

When used for poultry (Fig. 18), duckweed can be incorporated into broiler rations up to a proportion of 10% without compromising growth performance or skeleton composition (Nhapi, 2004). Higher ratios are reported to have negative effects on weight gain (Iqbal, 1999).

The literature contains different information on whether duckweed should be fed fresh or sun-dried: Edwards (2010) suggests fresh feeding because of rapid decomposition (UV light degrades valuable pigments) whereas Iqbal (1999) – without giving reasons – advocates dry feeding for chicken and Leng (1999) either fresh or dry administration.

Duckweeds of the *Lemna* and *Spirodela* genera may contain high amounts of Ca oxalate and therefore not be suitable for non-ruminant animals. Ruminants like cattle and sheep, on the other hand, ferment duckweed protein in the rumen, which possibly devalues the amino acids supplied to the animal. Nevertheless, weight gain in calves and sheep is reported to increase when their diet is supplemented with duckweed, and the taste of milk of dairy cattle is not affected by up to 75% duckweed feed (Iqbal, 1999).

Green Manure, Composting

Thanks to its high moisture and N content, duckweed can also be used as organic fertilizer and soil improver (Iqbal, 1999; Leng, 1999). It can either be applied directly or by way of composting and is reported to improve soil texture, water and cation exchange capacity (Iqbal, 1999).

Human Consumption

Duckweed could be a valuable food supplement in areas with malnourishment, particularly as a source of protein, essential amino acids, P, minerals and Vitamin A. However, its use for human nutrition is not widely practiced: only in certain regions of Southeast Asia have *Wolffia* species traditionally been cultivated and eaten by humans, most likely in areas and at times with otherwise limited availability of green vegetables (Leng, 1999). Possible explanations are the aforementioned Ca oxalate found in *Lemna* and *Spirodela* with its negative effects on health and taste and the difficulty to separate (pathogenic) organisms such as worms, snails, protozoa, and bacteria from the plant (Iqbal, 1999).

Mosquito Control

More and more evidence exists that duckweed can curb or completely eliminate mosquito development thanks to insecticidal compounds released by the plants (Leng, 1999). For instance, Marten et al. (1996) showed that *Anopheles albimanus* populations were negatively correlated with the amount of water surface covered by *Lemna*. In the wet tropics where malaria is again on the rise, duckweed aquaculture may therefore be one way to reduce the risk of transmission.

3.3.4 Constraints / Difficulties

Environmental / Seasonal / Ecological Constraints

In view of the climatic and nutrient requirements, extreme temperatures, high light intensity, wind, and a lack of water or nutrients can inhibit duckweed growth. In addition, duckweed is occasionally infested with insects (Edwards, 2010) but less sensitive to pests and diseases than other macrophytes (Iqbal, 1999). The main predators feeding on duckweed are herbivorous fish, snails, flatworms, ducks and other birds (Leng, 1999).

Algal blooms can repress duckweed if coverage of the surface is low and allows light to penetrate into the water. The algae become entangled with the roots of the duckweed plants whose fronds then turn

yellow and die (Iqbal, 1999). A combination with other floating aquatic plants is suggested to prevent the growth of algae and other plants that grow immersed in water (Leng, 1999). A mixture of plants also reduces susceptibility to diseases and pests compared to a monoculture (Zirschky and Reed, 1988).

Economic / Cultural Constraints

Cultivating duckweed is a continuous process and a highly intensive farming method with considerable labor inputs. It requires large areas of land (Edwards, 2010) – about 2-3m^2 per inhabitant if duckweed is grown on wastewater (Iqbal, 1999). The returns are low when weighed against required land, so that its production is not economically attractive unless land and labor costs are low and the market price for duckweed (or fish fed on it) high (ibid.).

Furthermore, if fresh duckweed cannot be used immediately, it either needs to be dried or transported to a place with foraging animals or fish farms. For large-scale duckweed cultivation there are so far no economically viable storage, drying, pelleting or ensilaging technologies (ibid.).

From a cultural perspective, aquaculture might be rejected in places where it has not traditionally been practiced.

Health Issues

When duckweed is grown in wastewater containing excreta, there is a potential risk of pathogen and helminths transfer to the human food chain. However, health risks are unknown when duckweed is used indirectly, e.g. as feed for fish, poultry, and mammals (Iqbal, 1999). Adequate protection measures as stated in chapter 2.4.3 (Public Health Concerns) are recommended all the same.

Duckweed has the capacity to accumulate metals such as Cr, Mn, Fe, Co, Cu, Zn, Cd, Pb, Al and even Au (Leng, 1999; Iqbal, 1999). This can be advantageous when duckweed is used for the purpose of phytoremediation of (industrial) waters loaded with heavy metals or to indicate potential pollution levels of water bodies. A low level accumulation in duckweed can be a valuable source of trace minerals for fish and livestock (Leng, 1999) but high level concentrations have detrimental effects on the plant itself as well as on animals or humans feeding on it. More than 800ppb Cd, for instance, prevent vegetative reproduction of duckweed (ibid.). As for *Azolla*, natural water sources are unlikely to cause problems but care must be taken with industrial wastewaters.

3.3.5 Previous Research

Duckweed has been extensively treated in the literature and used in practice to decrease water pollution, increase the potential for water re-use and produce biomass, mainly for feed purposes (Leng, 1999). The findings from selected studies and projects are presented below:

Nhapi (2004) declares duckweed systems to be an ideal method of natural wastewater treatment for developing countries because they “demand less in terms of financial resources for construction and maintenance, manpower sophistication, electricity requirements, and machinery.” Skillicorn et al. (1993) also state that duckweed-based wastewater systems could provide a genuine low-cost solution to problems of urban and rural human waste management. Especially in developing countries it should remain a decentralized approach, though, for if nutrients are exported from rural areas to a central treatment

site, the cost of transporting the biomass back to the farm where it is used would be extremely high (Leng, 1999).

Duckweed ponds for fodder production using manure from pigs, poultry, ducks, ruminants, and sometimes even human excrements as fertilizer are known from various Asian countries (Leng, 1999). In Bangladesh, the aforementioned NGO PRISM has been operating a duckweed-fish-production system since 1989 for the treatment of domestic sewage. Duckweed is harvested daily and fed to fish in adjacent ponds. The sale of fish leads to a yearly net profit per ha almost doubling that of rice – probably the first system generating profit from the treatment of domestic sewage (UNEP/GPA et al., 2004).

Research conducted on waste-fed duckweed over the past three decades illustrates that, among many other benefits, the plants provide efficient wastewater treatment through shading the water column and nutrient uptake (Edwards, 2010). Studies in Niger show that duckweed ponds for wastewater treatment can reach N and P removal efficiencies of about 80% as well as excellent pathogen removal of 4 log units, making the effluent suitable for reuse in agriculture (Seidl et al., 2004). If water is not a limiting resource, Rodriguez and Preston (1996) also consider the construction of duckweed ponds to be the most appropriate way to treat effluent from biodigestion of livestock wastes.

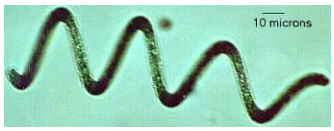
Lemna has already been used to treat sewage lagoons in the U.S.A., Europe and Australia (Leng, 1999). In industrialized countries, duckweed is particularly interesting due to its potential to clean industrial wastewaters through bioaccumulation. Although such systems are still expensive because high technology is needed to ensure success in treatment, both chemical and microbiological treatment plants appear to be much more costly (ibid.).

An ongoing study on duckweed for fuel ethanol production has already been presented in chapter 2.4.2; research in that field is certain to continue.

Summary and Conclusions for Field Experiment

An overview of the key findings of the above chapters is presented in Table 5:

Table 5: Summary of key data on spirulina, *Azolla*, and duckweed

	Spirulina *Arthrospira platensis*	***Azolla*** *Azolla caroliniana*	**Duckweed** *Spirodela polyrrhiza*
			
Requirements			
Temperature (survival / optimum [°C])	10-45 / 30-35	0-40 / 20-30	6-33 / 20-30
Light (photoperiod·day^{-1}; intensity)	10 hrs; direct sunlight (with agitation, temperature permitting)	14-16 hrs; direct sunlight	shade preferred
Nutrients	• all essential plant nutrients + HCO_3^- --> a distinct mixture to achieve desired salinity and pH; • NH_3 > 30mg·L^{-1} is toxic	• all essential plant nutrients + Mo or Co for N_2 fixation • N fertilizers stimulate competing plants • P = most common limiting element • salt content ≤ 0.3%	• all essential plant nutrients (incl. S) • N > 50-60mg·L^{-1} at a pH > 8 is toxic • N and P < 4mg·L^{-1} --> no significant nutrient reduction • P = main limiting nutrient
pH (survival / optimum)	~9-11.3 / 9.5-10.5	3.5-10 / 4.5-7	5-9 / 6.5-7.5
Other needs	agitation		
Use of Biomass			
Main use	human consumption (highly beneficial!)	green manure in paddy fields	animal feed
Secondary uses	animal feed	animal feed, human consumption	composting, biogas, human consumption
Constraints / Difficulties			
Environmental / seasonal	• suboptimal temperature and light • continuous culture • limited availability of nutrients (C, N, P,...) • risk of NH_3 toxicity • contamination with other algae, mud, bacteria	• intolerance of high heat and humidity • water availability throughout the year for nursery stocks • possible insect damage	• intolerance of extreme temperatures, high light intensity, wind • algal blooms when duckweed coverage is low • occasional insect infestation
Economic / cultural	• labor-intensive (agitation, monitoring, fertilization, harvesting, drying, processing) • costly fertilizer inputs • if grown on wastewater / urine uncertain acceptability for human consumption	• traditionally only cultivated in a few regions of Asia • lack of awareness of benefits • labor-intensive management compared to commercial fertilizers	• labor-intensive farming method, continuous process • large land requirements (low returns) • fresh duckweed must be used quickly, otherwise expensive drying / transportation • possible rejection of aquaculture where not traditionally practiced
Health	• potential contamination with heavy metals / toxic cyanobacterial compounds • necessity of hygienic processing	• possible contamination with pathogenic organisms • potential accumulation of heavy metals	• possible contamination with pathogenic organisms • potential accumulation of heavy metals

Previous Research

Regarding the use of urine / wastewater for production	• urine / animal wastes can substitute some nutrients but additional fertilizer inputs are needed • possible reduction of production cost through waste reuse • potential of integrated systems for nutrient recycling • questionable removal efficiency (NH_3 volatilization, PO_4 precipitation)	• successful cultivation on secondary treated domestic sewage, biogas residue, excreta-fed ponds • removal rates vary and depend on concentration of nutrients in production tank • fixation of atmospheric N_2 vs. removal from growing medium	• age-old practice of duckweed cultivation on animal / human excreta • natural low-cost wastewater treatment (esp. for developing countries) • good removal of N, P, and pathogens reported • potential to clean industrial wastewaters through bioaccumulation • duckweed for fuel ethanol

Spirulina

In view of the specific climatic requirements of spirulina and the actual temperature conditions of Kathmandu (cf. Fig. 40, chapter 5.4.1), it was decided that spirulina was not a suitable species for outdoor experiments in the winter half year. Although spirulina doubtlessly offers great nutritional benefits, cultivation in a suboptimal climate would require considerable additional inputs in order to assure productivity or even just survival of the culture (e.g. construction of a greenhouse, heating devices, constant energy supply). The exploration of such options was beyond the scope of this thesis because the core objective was to assess the suitability of urine or struvite production effluent as fertilizers and nutrient removal capacities of spirulina.

Another major factor that might inhibit the success of spirulina production in Nepal is the likelihood of social and cultural inhibitions towards consuming a product that has grown on urine. This leads to the question whether the high labor requirements (agitation, monitoring, fertilization, etc.), and additional costs of spirulina production (e.g. for supplementary fertilizers) would be well-received and justified if the product was then only used as animal feed. Though also beneficial as such, it remains to be investigated whether farmers or other entrepreneurs would be willing to invest time and money into a new crop whereas other sources of fodder are available at a lower expense.

Azolla and Duckweed

The requirements of *Azolla* and duckweed and the review of previous research about their use in wastewater treatment indicated that both species might be successfully grown on diluted urine and struvite production effluent. The early spring season in Nepal would not provide optimal climatic conditions but still be within the tolerance range of the plants.

Since the literature describes P as a major growth limiting factor, the plants were assumed to be restrained on P-stripped effluent, which was to be accounted for during the experiment by a control treatment with added P fertilizer (s. growing media, chapter 4.2).

While *A. caroliniana* abounded in several ponds in and around Kathmandu, the scarcity of duckweed inoculum proved to be a problem: though said to be ubiquitous during the rainy season (Thapa, 2009), it was in short supply at the end of winter and early spring in Kathmandu. A colony could finally be found in a neighboring town, and after its identification as *Spirodela polyrrhiza* at the Herbarium of the Botanical Gardens in Godavari, the species was chosen for the experiments.

PART II: FIELD EXPERIMENTS

4. Methodology

4.1 Experimental Site

4.1.1 Location and Site Preparation

The field experiments were carried out in Siddhipur (cf. STUN-Project, chapter 2.3). The experimental plot was located on a largely organic farm owned and run by Jiban Maharjan at the edge of the village, surrounded by vegetable fields and near a gravel road with little traffic.

The locality was chosen because of its previous involvement with the STUN-Project (see chapter 2.3), the existing urine collection system, the availability of a suitable plot of land, sufficient water, and the unlikelihood of disturbance by local residents.

Fig. 19: 500-l water tank used to fill experimental tanks

Before setting up the experimental tanks, the ground was leveled and the top bars of a nearby wooden fence removed to prevent uneven shading. To facilitate water access, a 500-L polypropylene tank with a tap was installed at a small distance and filled with well water through a pipe (Fig. 19).

4.1.2 Production Tanks

Black 100-L polypropylene (PP) drums of 47 cm diameter (tank surface 0.1735m^2) were bought at a local household supply store and cut with a heated metal saw at a height of 29cm to form open tanks (Fig. 20). This height was chosen to allow for a rim of about 8cm above the surface so as to minimize turbulence due to wind, which could have adversely affected *Spirodela* (cf. chapter 3.3.2). The tanks were placed on the experimental plot spaced about 40cm apart to avoid mutual shading. In order to facilitate monitoring and readjustment of the water level, marks were placed with whiteout on the interior walls of the tanks at 20, 25, 30 and 35 liters.

Fig. 20: Cutting experimental tanks

PP drums were preferred to other structures such as cement rings, tarpaulin or welded metal basins due to their comparatively low price, suitable size and stability, easy availability, transport and installation, as well as the reusability or recyclability of the material at the end of the trial. Besides, the black colored PP was thought to absorb more solar radiation and heat up the growing media to favorable temperatures more quickly on the cool winter mornings at the beginning of the experiment. Compared to transparent aquariums – which were not shortlisted as possible containers due to the high risk of breakage –, black side walls also prevent lateral incidence of light, thereby curbing algae growth.

4.2 Growing Media

Three different types of growing media were used: (1) diluted urine, (2) diluted struvite production effluent as well as a control treatment with (3) diluted effluent and added diammonium phosphate (DAP) to determine if P is the growth-limiting factor when P-stripped effluent is used as growing medium.

4.2.1 Urine

Source

Urine was collected from 12 households in Siddhipur having EcoSan urine separation toilets. It was stored in jerrycans and PP tanks at Jiban Maharjan's farm. Some of the storage containers held urine from mixed sources, others from a single household.

Analysis

Due to its varying composition, storage time and nutrient content, the urine to be used was analyzed for NH_4-N and PO_4-P before fertilizing the production tanks at the beginning of the experiment and on a weekly basis thereafter to determine the volume needed for the desired nutrient concentration.

The initial sample preparation and nutrient analysis was carried out by ENPHO in Kathmandu where the urine was tested, among others, for

- nitrate (NO_3^-; UV spectrophotometric, screening),
- ammonium (NH_4^+; spectrophotometric, Nesslerization),
- orthophosphate (PO_4^{3-}; ammonium molybdate ascorbic acid red.),
- potassium (K^+; atomic absorption spectrometer, flame emission),
- *E. coli* (membrane filtration, CFU per 100ml).

The results (see Table 19, Appendix A) were used to calculate the required initial urine quantity to be added to the experimental tanks.

Due to the decomposition of urea and urate, stored urine contains N mostly (>90%) in the form of ammoniacal N and its compounds (Kirchmann and Pettersson, 1995). ENPHO results confirmed that nitrate (NO_3^-) only accounted for 0.57% of N present in the analyzed urine sample (Table 20, Appendix A). It was therefore considered sufficient to focus on NH_4-N for further analyses and neglect the small amount of NO_3^- present because it would not be a significant nutrient source for the plants.

Subsequent NH_4-N and PO_4-P analyses were done by the author at the UN-Habitat office in Patan, using a Hach DR/2000 photospectrometer. For a detailed description of sample preparation and analysis see chapters 4.8.2 - 4.8.4. K content was determined at the Eawag laboratory in Dübendorf, Switzerland; details thereof are described in chapter 4.8.5.

4.2.2 Struvite Production Effluent

Production

The struvite reactor especially designed by STUN for community scale struvite production and installed at Jiban Maharjan's farm was used to obtain effluent for the experiments (Fig. 21). The struvite reactor

was run three times throughout the experiment, processing a volume of 10 L of urine each time. The urine was poured into the steel drum and mixed with 30g of $MgSO_4$ (Rohit Chemicals, Birganj, Nepal). Since the reactor had been designed for 50 L, the blades of the stirring mechanism did not reach the urine so that a wooden stick was used for manual stirring instead. After 10 minutes of stirring the struvite was left to settle for 15 minutes, whereupon the tap at the bottom of the reactor was opened to let the liquid drip through a nylon filter bag of <100µm pore size (Etter, 2009). Precipitated struvite remained in the bag and the effluent was caught in a bucket underneath. It was later transferred to an airtight jerrycan until use to minimize NH_3 volatilization.

Fig. 21: STUN struvite reactor, Siddhipur (Etter 2009)

Analysis

Effluent was analyzed by ENPHO in the same way as urine, see chapter 4.2.1 above.

4.2.3 Effluent + Diammonium Phosphate (DAP)

Source

Effluent was produced, stored and analyzed as described above (chapter 4.2.2). DAP fertilizer was available from Jiban Maharjan who had purchased it from a local fertilizer store.

Preparation of DAP Solution

In order to make DAP easy to dose and measure its soluble P-content, 10g DAP granules were dissolved in 1000ml well water. NH_4-N and PO_4-P content of the solution was measured with a Hach DR/2000 photospectrometer (detailed method chapter 4.8.3 and 4.8.4) after 2 and 24 hours, producing the same results. 2 hours were therefore considered ample dissolution time for the two further preparations of DAP solution in the course of the experiment. The solution was stored in PET bottles until use. After each fresh preparation, NH_4-N and PO_4-P content was measured anew (see result sheet Table 23, Appendix C).

4.2.4 Water

Supply

Water was available throughout the experiment from a dug well on Jiban Maharjan's farm.

Analysis

The water was analyzed at ENPHO for physico-chemical parameters, including NH_4^+, NO_3^-, ortho-PO_4^{3-} and K. All tested parameters were within the National Drinking Water Quality Standards (see analysis report Table 21, Appendix B) and were not thought to distort the results of the experiment.

Two water samples of 50ml each were acidified with nitric acid (HNO_3) to a final acid concentration of 1% (Udert, 2010) and analyzed at Eawag in Dübendorf, Switzerland, for heavy metals and micronutri-

ents (Na, Mg, Al, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd) to get an idea of potential deficiencies or accumulations of these elements, which could limit plant growth and/or make the biomass unsuitable for further agricultural use. At Eawag, the samples were filtered and analyzed undiluted with ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

4.2.5 Dilutions

The urine and effluent were diluted with well water to a concentration of 20mg·L^{-1} NH_4-N in order to meet plant requirements (cf. chapters 3.2.1 and 3.3.1) and acknowledge the results of a previous study with species of the same (sub)families (*Azollaceae*, *Lemnoideae*) (Marti, 2000) where such concentrations had produced peaking growth and biomass production rates. N was chosen as benchmark parameter across the treatments because it was the target nutrient to be removed from urine and effluent by the plants. To reach the desired concentration, between 125 and 145fold dilution was required. (Average NH_4-N concentrations in urine and effluent lay around 2400mg·L^{-1}, cf. Appendix C.)

In the DAP-fertilized control treatment the PO_4-P level was increased to 4mg·L^{-1} with DAP solution. A P:N-ratio of 1:5 was aimed at because it corresponds to the approximate chemical composition of *Azollaceae* and *Lemnoideae* (Xavier et al., 1990) and was considered to provide optimal growth conditions.

4.3 Aquatic Plants Used

4.3.1 *Azolla caroliniana*

Azolla inoculum was collected from the community pond in the village of Imadol between Kathmandu and Siddhipur (see map, Fig. 5) where a thick and dense cover of *Azolla* coated the entire pond surface (Fig. 22). The upper layers showed red colored lobes, possibly due to P deficiency or sun exposure (cf. chapter 3.2.2). The inoculum was taken to the experimental site and transferred into preculture tanks within two hours.

Fig. 22: *Azolla* source: community pond, Imadol

4.3.2 *Spirodela polyrrhiza*

Spirodela inoculum was collected from a small pond near the Mahakali temple in Bhaktapur, a town 15km east of Kathmandu (see map, Fig. 5). The pond's surface being sparsely strewn with *Spirodela* (Fig. 23), the plants were filtered from the water with a metal sieve. The inoculum was taken to the experimental site and transferred into preculture tanks within two hours.

Fig. 23: *Spirodela* source: pond near Mahakali Temple, Bhaktapur

4.4 Timeline of the Trial

The field trial took place from mid-January to mid-March and was split into a 2-week preculture period, a 3-week long preliminary experiment and a 3-week long main experiment. The preliminary experiment served the purpose of improving the experimental set-up as well as tank management and analytical skills. The results and discussion part of this thesis (chapter 5) focuses exclusively on the main experiment. For an overview of the different phases and activities, refer to Table 6 below:

Table 6: Overview experiment – timeline and activities

Date		January		February				March		
		week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10	week11
Activities	urine/effluent/DAP analyses	Temperature measurements: Mo, Mi, Fr of each week								
		inoculation fertilization	urine/effluent analyses	inoculation fertilization	biomass weighing sample analyses urine/effluent analyses fertilization	biomass weighing sample analyses urine/effluent analyses fertilization	biomass weighing sample analysis *Spirodela* samples for total N analysis	inoculation fertilization (staggered 2/3 + 1/3)	sample analyses urine/effluent analyses fertilization (staggered 2/3 + 1/3) 24-hour temp. measurement	sample analyses urine/effluent analyses fertilization (staggered 2/3 + 1/3)	biomass weighing sample analysis *Spirodela* samples for total N analysis
		preculture		**preliminary experiment**				**main experiment**		

4.5 Preculture

The preculture consisted of two tanks as described above for each *Azolla* and *Spirodela*. The tanks were filled with well water to a volume of 35 L, corresponding to a depth of approximately 20cm, in accordance with suggestions found in the literature (Iqbal, 1999; Metcalf and Eddy, 2003).

The inoculum was washed to get rid of excess mud and algae; it was then sorted through by hand to remove snails, insect larvae and foreign plants. Regarding *Azolla*, individuals with only minor signs of red coloration were selected to minimize initial P-deficiency. Only about half of the tank surface was covered with inoculum (approx. 30g *Spirodela* and 50g *Azolla* per tank) to allow for reproduction over a preculture period of 2 weeks.

The tanks were fertilized with stored human urine and dissolved DAP granules to obtain a calculated concentration of 20mg·L^{-1} NH_4-N and 4mg·L^{-1} PO_4-P (sample calculation in chapter 4.8.6). The fertilizer was added only after plant inoculation to reduce NH_3 volatilization from the open water surface. In order to keep the volume at 35 L, the equivalent amount of water was removed before fertilization.

4.6 Experimental Set-up

4.6.1 Experimental Design

The three different fertilization treatments and two plant species resulted in 6 different settings:

- *Azolla* / Urine
- *Azolla* / Effluent
- *Azolla* / Effluent + DAP
- *Spirodela* / Urine
- *Spirodela* / Effluent
- *Spirodela* / Effluent + DAP

Fig. 24: Set-up of experimental tanks, randomized design

Each setting had three replications, totaling 18 tanks, for which a randomized set-up was used (Fig. 24). An additional tank was put up to measure evaporation.

4.6.2 Start of the Main Experiment

Filling of Tanks

The tanks were filled, inoculated and fertilized as described for the preculture (chapter 4.5) to obtain the desired dilutions (chapter 4.2.5). Since the supply tank did not hold enough water to fill all 18 experimental tanks at once, they were filled in two steps, on the 1st and 3rd day of the experiment, adding 20 and 15 liters, respectively. In between, the supply tank was refilled with well water by pipe and mud particles that had been swirled-up from the bottom were left to settle for two days. This was done to avoid contamination of the experimental tanks with excessive suspended solids, which had happened during the preliminary experiment where filling was continued immediately after the supply tank had been refilled, leading to different starting conditions in the experimental tanks.

Inoculation and Protection of Biomass

The preliminary experiment showed that 10g *Azolla* and *Spirodela* inoculum per tank (= 55-60g·m^{-2}, following van Hove et al., 1987; Kamalasanana Pillai 2008; Marti, 2000) did not cover enough of the surface to produce sufficiently strong populations, and the macrophytes were eventually outcompeted by algae.

For the main experiment, inoculation therefore comprised higher quantities of plant material to give *Azolla* and *Spirodela* a head start over algae. *Azolla* tanks were inoculated with 60g fresh material, covering approximately 60% of the tank surface (suggested by Graber, 2010). *Spirodela* tanks were inoculated with 22g fresh material, based on the fact of limited availability.

During the preliminary experiment an increasing number of crows were witnessed near or even on the tank rims. The tanks were henceforth covered with rows of chicken wire (Fig. 25) to prevent biomass loss through foraging animals.

Fig. 25: Experimental tanks covered with chicken wire

Initial Fertilization

To maintain even nutrient concentrations, the initial fertilizer volume was divided into two doses of 2/3 and 1/3, applied on the 1st and 3rd day of the experiment.

4.6.3 Fertilization Regime

After the weekly nutrient removal measurement (see chapter 4.8 below), the growing media were adjusted to the initial volume (35 L) with well water and fertilizer – urine, effluent, effluent + DAP –, the volume of which was calculated to keep the nutrient concentrations close to 20mg·L^{-1} NH_4-N (and 4mg·L^{-1} PO_4-P for the DAP-fertilized tanks). Since nutrient removal varied among treatments and plant species, the fertilizer volumes were calculated separately for each setting, based on the median values of the three replications (sample calculation in chapter 4.8.6).

Addition of water and fertilizer was done in the mornings on the day after sampling. During the preliminary experiment the tanks were replenished in a single go; during the main experiment the fertilizer volume was divided into two doses of 2/3 and 1/3, applied on two consecutive days. Split application, especially of P fertilizer, has been reported to yield maximum biomass production without stimulating competing organisms such as green or blue-green algae (Hamdi, 1982).

4.7 Biomass Production Measurement

4.7.1 Fresh Weight

During the preliminary experiment, biomass production was measured by weighing the plant material of each tank after 10, 17 and 24 days, using an electronic kitchen scale with a high precision strain gauge sensor system (1g increments). The plants were harvested from the surface with a plastic beaker and drained for 5 minutes on a nylon sieve (0.75mm mesh size) attached to a specially designed metal ring with three supporting rods (Fig. 26). The growing medium could thereby drip back into the experimental tanks. The plants were returned to the tanks after weighing, distributed on the surface and turned face up as far as possible, which did not happen automatically in the case of *Azolla*.

Fig. 26: Harvesting plants for weighing

However, the weighing process appeared to have detrimental effects on the plants. During the main experiment, they were consequently only weighed once at the time of inoculation and again at the end of the experiment (after 22 days) to minimize stress and physical damage inflicted on them.

4.7.2 Dry Matter

The conversion factor fresh/dry matter was assessed in order to make the results comparable to values stated in the literature (e.g. Iqbal, 1999; Leng, 1999; DeBusk and Reddy, 1987) and calculate the actual amount of N taken up by the plants. It was only determined for plants produced during the main experiment.

Following Marti's (2000) procedure with plants of the same (sub)family, a fresh biomass sample from each of the six settings was dried for 12 hours at 105°C, which was done in an oven at the ENPHO lab where back-up batteries could be used to bridge several hour-long power cuts.

The samples were made up of 10g plant material from each replicate, totaling 30g per sample. They were placed in porcelain dishes and their weight recorded. After drying, the dishes were put in a desiccator for 1 hour to cool down and then weighed again to determine the specific conversion factor for each setting. This factor was then applied to calculate dry matter production for each tank.

4.7.3 N-Content of *Spirodela*

A biomass sample of *Spirodela* from each of the three different treatments was analyzed for its total N content to get an idea what proportion of the recorded N-removal from the tanks had actually been transformed into the desired biomass and which had been lost otherwise (e.g. through volatilization). *Azolla* was not suitable for this analysis because of its capacity to fix atmospheric N_2 (cf. chapter 3.2.1).

The samples were made up of 20g plant material from each replicate, totaling 60g per sample. The biomass was put in sealable plastic bags and transported to Kathmandu University (KU) in Dhulikel the following day where they were stored in a fridge until analysis. Total N analysis was carried out by the Aquatic Ecology Center Laboratory of KU according to procedures based on digestion of plant material in a sulfuric-salicylic acid mixture (Icarda, 2010).

4.8 Nutrient Removal Measurement

4.8.1 Sample Collection

Growing medium samples were taken after the first, second, and third week of the preliminary as well as the main experiment. The samples were collected around 9:30 am in 50ml Greiner sampling flasks after mixing the experimental tanks with a wooden stick. To minimize cross-contamination and transmission of plants between the tanks, the stick was wiped dry after each use. The samples were then transported to the UN-Habitat office in Patan and photospectrometric analyses were performed the same day.

4.8.2 Sample Preparation and General Laboratory Procedures

Prior to nutrient measurements, the urine samples were filtered in two steps with 0.7 and 0.45µm Millipore and Whatman filter papers (Tilley, 2007; Udert, 2010) in a filter syringe to remove solid contaminants that could have caused interferences. DAP and all growing media samples were directly filtered with 0.45µm filter papers unless suspended algae required prior filtration with larger pore size. Due to a shortage of 0.45µm filters, the samples were filtered through 0.7 and 0.2µm filter papers for the last measurements (week 11).

The unavailability of distilled water due to power shortages made it necessary to replace it with a comparable alternative from early March (week 9) onwards. Two brands of bottled drinking water and distilled water intended for car batteries were tested for their NH_4-N and PO_4-P content; car battery water was considered most suitable due to its low NH_4-N values. After being used for one series of analyses in week 9, it turned out to be of questionable quality, though: a strong chlorine-like smell was discovered and the obtained NH_4-N results were improbably low. Since residual chlorine is known to react with NH_3 and create possible interferences (Standard Methods, 4500-NH_3 Nitrogen (Ammonia), APHA et al., 2006)), the car battery water was abandoned in favor of Aqua Hundred bottled drinking water.

Measuring cylinders were always rinsed three times between use with distilled water (mineral water, respectively); photospectrometer vials with tap water. Sampling bottles were rinsed three times with tap water and left to dry in the sun.

4.8.3 Ammonium (NH_4-N)

For NH_4-N analyses, the salicylate method no. 8155 (Hach, 1996) was used. In order to match the measuring range and take recommendations by Tilley (2007) into account, the urine, effluent, and DAP solution samples were diluted 1:10'000 with distilled water, which was done in two steps (1:100, 1:100) using 50ml measuring cylinders. Growing media samples were diluted 1:100.

After the first series of analyses during the preliminary experiment produced extremely scattered and unlikely results, the recommended reaction time of 3 and 15 minutes for the powder pillows was doubled to compensate for the rather low ambient temperature (10-15°C). The doubled reaction time was maintained until the end of the experiment despite rising spring temperatures because spot checks showed that the measured values remained stable after reaction times of 6 and 30 minutes whereas changes still occurred with shorter reaction periods. In view of the number of samples to be analyzed and limited glassware to prepare them, a longer reaction time was also more practical to provide identical conditions for all samples.

To check the accuracy of the obtained results, a standard addition was carried out using Merck standard solution with a concentration of 1000mg·L^{-1} NH_4 (see Appendix F).

4.8.4 Phosphate (PO_4-P)

For PO_4-P analyses, the molybdovanadate method no. 8114 (Hach, 1996) for reactive P (ortho-PO_4^{3-}) was used. Urine and effluent were diluted 1:10, DAP solution 1:250. No dilution was necessary for the growing media samples. As for NH_4-N analyses, the reaction time was doubled from 3 to 6 minutes.

4.8.5 Potassium (K)

During the main experiment, weekly growing medium samples (~30ml) from one tank of each setting were kept, filtered as described above and acidified for later K analysis at Eawag in Dübendorf, Switzerland. According to the procedures manual for photospectrometric analysis of K, method no. 8049 (Hach, 1996), the samples were stabilized by adding HNO_3 (Qualigens Fine Chemicals) until pH indicator strips showed a pH between 1 and 2.

Likewise, samples of the urine used for fertilization were preserved with HNO_3 on a weekly basis because the composition and nutrient concentration varied. Since effluent was used from a single production time throughout the main experiment, it was sufficient to acidify one sample.

At Eawag, the samples were filtered and diluted; the growing medium samples 1:10, the urine and effluent samples 1:1000. Analysis was done by AA (Atomic Absorption).

4.8.6 Calculations

Fertilization

Initial fertilizer quantities (urine/effluent/DAP) and weekly replenishment requirements were determined according to the following two sample calculations (Table 7 and Table 8, based on values from the result sheets of NH_4-N and PO_4-P measurements in Appendix E):

Table 7: Sample calculation for initial fertilization *Azolla* / Urine tank (no.4; week 8)

	desired concentration [mg·L^{-1}]	desired volume in tank [L]	required absolute load [mg]	measured concentration in urine [mg·L^{-1}]	**required volume of urine** (requ.abs.load / conc.in urine) **[L]**
NH_4-N	20	35	700	2900	**0.241**

→ The tank was filled with 34.759 L of well water and 0.241 L of urine (approximation).

Table 8: Sample calculation for weekly fertilization of *Spirodela* / E + DAP tanks (week 10)

	measured concentration (median of tanks 6,9,14), before fertilization [mg·L^{-1}]	volume of growing medium in tank [L]	absolute load (conc.· volume) [mg]	desired concentration [mg·L^{-1}]	desired volume in tank [L]	desired absolute load [mg]	**difference (fertilizer need) [mg]**
NH_4-N	7	30.3	212	20	35	700	**488**
PO_4-P	1.6	30.3	48	4	35	140	**92**

	measured NH_4-N concentration [mg·L^{-1}]	measured PO_4-P concentration [mg·L^{-1}]	**added volume [L]**	**added NH_4-N [mg]**	**added PO_4-P [mg]**
effluent	2300	75	**0.133**	306	10
DAP	1700	775	**0.104**	177	81
			total:	**483**	**91**

→ The tanks no. 6, 9, and 14 were re-fertilized with 0.133 L of effluent and 0.104 L of DAP solution.

Nutrient Removal

Nutrient removal from the tanks was calculated based on

- urine and effluent analyses before the experiment and before weekly fertilization
- tank-specific added volumes thereof (→ added nutrient loads),
- measured concentrations of NH_4-N, PO_4-P, and K,
- volume present in the tanks at the time of sampling.

NH_4-N, PO_4-P, and K contents of the well water were not included in the calculations for fertilizer addition but were taken into account to determine total nutrient removal for each tank at the end of the experiment. The water volumes added to each tank were multiplied with the well water nutrient concentrations assessed by ENPHO (0.3339mg·L^{-1} NH_4-N, 0.039mg·L^{-1} PO_4-P, and 5.08mg·L^{-1} K; cf. Appendix B). A sample calculation for total nutrient removal is below (Table 9):

Table 9: Sample calculation for total NH_4-N removal from *Azolla* / Effluent tank (no. 7)

	initial fertilization week 8 [mg]	fertilization week 9 (added NH_4-N: 0.165 L effluent·2500mg·L^{-1}) [mg]	fertilization week 10 (added NH_4-N: 0.17 L effluent·2300mg·L^{-1}) [mg]	NH_4-N from well water (43.32 L water ·0.3339 mg·L^{-1}) [mg]	**total added NH_4-N [mg]**
NH_4-N	700	413	391	14	**1518**

	measured NH_4-N concentration week 11 [mg·L^{-1}]	volume in tank week 11 [L]	absolute load remaining in week 11 [mg]	**total NH_4-N removal [mg]**
NH_4-N	7	31.5	221	**1297**

→ Of 1518mg added NH_4-N, 1297mg were removed and 221mg remained in tank no.7 at the end of the experiment.

Biomass Production

Total biomass production over the three weeks of the main experiment was calculated based on

- fresh weight of plant material used for inoculation,
- fresh weight of total plant material collected from each tank at the end of the experiment,
- treatment-specific dry matter conversion factors.

Since *Azolla* plants were in different shape at the end of the experiment and *Spirodela* tanks contained varying proportion of algae depending on the growing medium, the dry matter conversion factors were determined separately for each treatment. Those plants in tanks fertilized with effluent and DAP looked optically closest to the plants used as inoculum – green color for *Azolla*; free from algae for *Spirodela* – so that the same conversion factor (E + DAP treatment for each *Azolla* and *Spirodela*) was also used to convert initial fresh weight plant material into dry matter. From there, the increase of biomass in % of inoculum could be assessed. A sample calculation for total biomass production is below (Table 10).

Table 10: Sample calculation for total biomass production in *Spirodela* / Urine tank (no. 17)

initial fresh weight [g]	dry matter conversion factor (E + DAP treatment)	initial dry weight [g]
22	0.0581	1.2782

final fresh weight [g]	fresh weight increase	treatment specific dry matter conversion factor	dry weight increase [g]	**biomass increase in %** (dry weight increase/initial dry weight)
92.5	70.5	0.0553	3.89865	**305%**

N Assimilation by Spirodela

The proportion of N assimilated by *Spirodela* was calculated based on

- dry matter increase of *Spirodela,*
- total N content in % of dry matter (results from analysis by KU, see Appendix D),
- comparison of total N present in *Spirodela* biomass with N removal from tanks.

A sample calculation for the ratio of N assimilated by *Spirodela* is below (Table 11):

Table 11: Sample calculation for proportion of N assimilated by *Spirodela* (*Spirodela* / Effluent tanks)

average dry matter increase per tank [g]	total N content of *Spirodela* (% of dry matter)	N assimilated by *Spirodela* [mg]	average N removed from tanks [mg]	**proportion of N taken up by *Spirodela***
3.0112	1.43%	43.06	1536	**2.80%**

4.9 Environmental Parameters

4.9.1 Temperature

Ambient and growing medium temperature were measured three times a week over the entire trial period in order to record seasonal changes. The measurement was done with a temperature probe integrated in an electric conductivity meter (WTW LF 340) between 9:15 and 9:45 am by first recording ambient temperature in the shade and then submerging the probe 10cm into each of the experimental tanks one by one until the display stopped changing. The probe was always submerged at the same spot in all tanks to minimize the variations attributable to different exposure to the sun within the tanks.

In addition, diurnal temperature fluctuations were measured over a 24-hour period on a typical unclouded and sunny day in early March (week 9). Between 7 pm and 7 am only 4 tanks and the plantless evaporation control tank were measured; the former had been chosen because their cumulative recorded temperature was closest to the cumulative temperature median of all 18 tanks.

To calculate average water temperature, only tanks with plants (no.1-18) were included. Standard deviation values were determined (in Excel) to characterize the differences among the tanks.

4.9.2 Humidity and Wind Speed

Relative humidity and wind speed were intended to be measured to assess whether they were in line with plant requirements. However, due to a shortage of time and difficult availability of suitable equipment the recording of these parameters was omitted.

4.9.3 pH

pH was recorded three times a week around 9:30 am with a probe (WTW 315i) immersed in the tanks as described for temperature measurements. Due to a defect of the probe display, measurements were stalled in week 9 and could not be taken during the 24-hour measurement of diurnal fluctuations.

4.10 Statistical Analysis

Biomass production and nutrient removal results were analyzed with the following statistical measures:

1) Standard errors to account for variations between replications of the same treatment and the reliability of the obtained average values (nutrient removal: expressed in absolute quantities [mg]).

2) The statistical significance (Excel Anova: Single Factor analysis) of
   - the difference in biomass production between species (*Azolla*/*Spirodela*), i.e. the probability that the difference in biomass dry matter increase (in % of inoculum dry weight) is dependent on the species and not caused by random variation.
   - the difference in biomass production between growing media, i.e. the probability that the difference in biomass dry matter increase (in % of inoculum dry weight) is dependent on the fertilizers (urine/effluent/E+DAP). This analysis was done separately for *Azolla* and *Spirodela*.
   - the difference in nutrient removal between species (*Azolla*/*Spirodela*), i.e. the probability that the difference in NH_4-N and PO_4-P removal is dependent on the species. K removal was not included due to limited data (measurements of only one replication per treatment).
   - the difference in nutrient removal between growing media, i.e. the probability that the difference in NH_4-N and PO_4-P removal is dependent on the fertilizers (urine/effluent/E+DAP). This analysis was done separately for *Azolla* and *Spirodela*.

   The analysis for nutrient removal was based on absolute quantities [mg] and not removal rates [%].
   $\alpha < 5\%$ was set as target for significant differences; $\alpha < 1\%$ for very significant differences.

3) Correlations between nutrient removal (NH_4-N, PO_4-P, K in [mg]) and biomass production (*Azolla*/*Spirodela* dry matter increase in %), i.e. if total nutrient removal shows a relationship with biomass production (Excel correlation function) and visualization in a diagram with data points.

5. Results and Discussion

Results and discussion are grouped in the same chapter to facilitate referencing and orientation for the reader and to minimize repetitions. In each subchapter, the obtained results are first presented factually, e.g. with a description, table or graph, and where pertinent a statistical analysis. They are then discussed and compared with the literature in subsequent paragraphs.

5.1 Biomass Production and Analysis

5.1.1 Fresh Weight

Fresh weight biomass production reached values between 256 and 301g for *Azolla* and between 67 and 86g for *Spirodela* (Fig. 27), corresponding to multiplication factors between 4.23 and 5 for *Azolla* and between 3.05 and 3.9 for *Spirodela* within the experimental period of 22 days. For both plant species, average biomass production was highest in the tanks with urine as a growing medium and lowest in those with effluent and DAP. Standard errors amounted to 7g, 1g and 6g for *Azolla* and 3g, 3g and 6g for *Spirodela* in the three different growing media (urine, effluent, effluent + DAP).

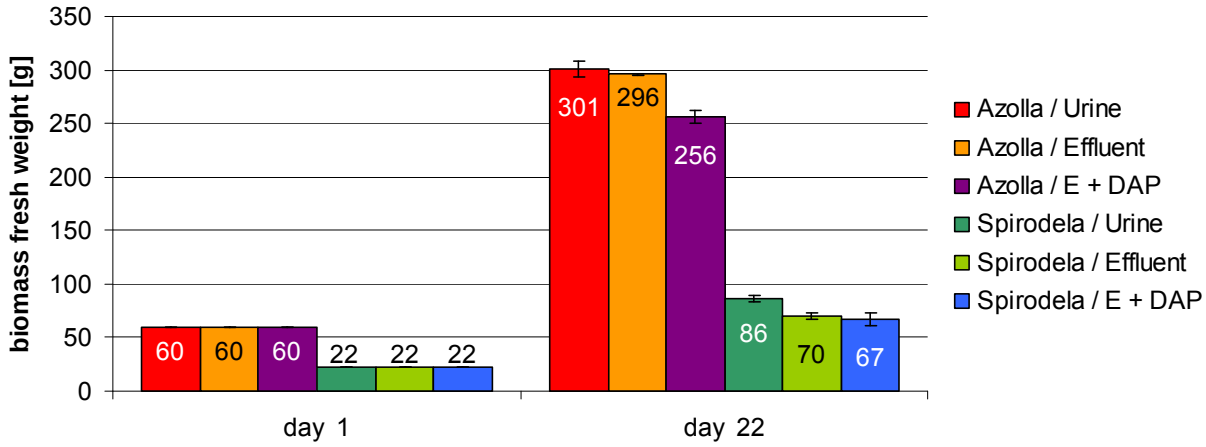


Fig. 27: Biomass production (fresh weight)

Fresh weight biomass values are difficult to compare with the literature where mostly dry weight is stated. One author (Kamalasanana Pillai, 2008) who mentions fresh biomass yields for *Azolla* writes of a production of 300g·m^{-2}·d^{-1} which would be 52.05g·d^{-1} in a tank of the size of those used in the experiment (0.1735m^2 surface area). This value lies clearly beyond what was produced in the experimental tanks. If biomass were assumed to have grown linearly, even the most productive treatment (*Azolla* / Urine) only produced 13.6g·d^{-1}. Standard errors were small and indicate that the average biomass production would not vary substantially were the experiment repeated under the exact same conditions. A more extensive discussion of biomass production follows in chapter 5.1.2 on dry matter.

5.1.2 Dry Matter

The conversion factors from fresh to dry matter (DM) for each treatment were established as described in chapter 4.7.2 and turned out as follows (Table 12):

Table 12: Dry matter conversion factors

treatment	conversion factor
Azolla / Urine	4.44%
Azolla / Effluent	4.95%
Azolla / E+DAP	4.31%

treatment	conversion factor
Spirodela / Urine	5.53%
Spirodela / Effluent	6.23%
Spirodela / E+DAP	5.81%

The conversion factors lay between 4.3 and 5% for *Azolla* and 0.5 to 1.5 percentage points higher for *Spirodela*. The determined values for *Spirodela* were slightly lower than those in the literature where a dry weight fraction between 6-8% is reported (Gijzen, 1997; Iqbal, 1999). A possible reason for this could be that – in the case of *Spirodela* – the harvested and dried plant material also contained some algae with higher moisture content.

A comparison of absolute biomass weight (DM in grams) poses the problem that different amounts of inoculum were used for *Azolla* and *Spirodela*. It is therefore more applicable to compare DM increase in % of initial plant material (also converted into DM), which has been done in Fig. 28:

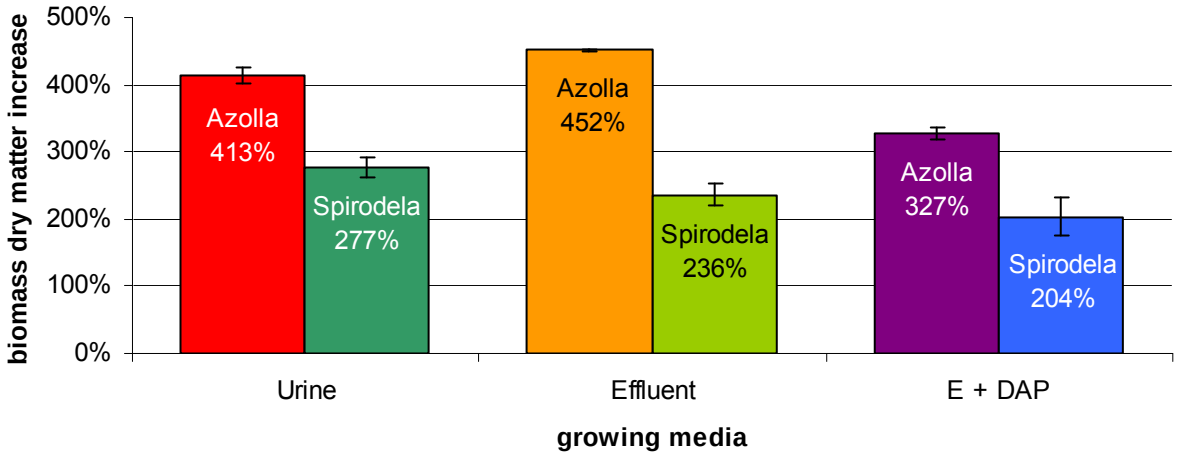


Fig. 28: Percent increase of inoculum, measured in terms of dry matter

In all three growing media, *Azolla* showed higher biomass increase than *Spirodela*. *Azolla* production peaked in effluent fertilized tanks with 452% increase and was lowest in the tanks with effluent + DAP with 327% increase. *Spirodela* reached its maximum in the urine fertilized tanks and its minimum also in those with effluent + DAP. The difference in performance between *Azolla* and *Spirodela* in terms of biomass production was most apparent in the tanks fertilized with effluent. Standard errors in *Azolla* treatments ranged between 1% (*Azolla* / Effluent) and 13% (*Azolla* / Urine); in *Spirodela* treatments they were higher with between 14% (*Spirodela* / Urine) and 27% (*Spirodela* / E + DAP)

The reasons for *Azolla*'s clearly more vigorous growth can be manifold: higher inoculation density and therefore less competition from algae, capacity to fix additional N_2 from the atmosphere, longer roots to penetrate into deeper levels and absorb more (micro) nutrients. The latter, however, stands in contrast to observations by Leng (1999) that the addition of N fertilizer in aquatic media removes the major advantage of *Azolla*, that is its ability to grow on waters low on N. Low quantities of inoculum are more

likely to have put *Spirodela* at a disadvantage: unlike other authors who mention inoculation densities around 60g·m^{-2} (cf. chapter 4.6.2), Leng states that its growth potential is limited below 600g·m^{-2} fresh biomass. *Azolla*, on the other hand, is reported to reach greatest productivity at intermediate plant density levels, though no precise value is given (DeBusk and Reddy, 1987).

One of the causes for *Spirodela*'s modest biomass production may also lie in the fact that rapid growth only commences when protein content has arrived at the highest level – which appears to be three weeks after introduction into nutrient-rich waters (Leng, 1999). *Spirodela* might therefore not have reached its peak productivity within the duration of the experiment. Moreover, *Spirodela* may have suffered from exposure to direct sunlight which has been recognized to increase death rates (ibid.), whereas *Azolla* tolerates or even prefers such conditions (cf. chapter 0).

With regard to the different growing media, *Azolla* surprisingly showed highest biomass production in the effluent fertilized tanks despite low P concentrations, and *Spirodela* in effluent also had growth rates exceeding those of the tanks with added DAP. Duckweed is known for its capacity to take up nutrients (N and P) from wastewater as long as they are available and store them in its tissue. Once N and P are completely removed from the water, the plants can then use their internally stored nutrients and keep growing for a significant period of time (Cheng and Stomp, 2009b). It is plausible that *Azolla* has a similar strategy to provide for times with limited nutrient supply, relying on accumulated P when concentration in the growing medium is insufficient. However, in the long run a shortage of P is anticipated to inhibit growth, and mere biomass production values do not reveal much about the health status of the plants. In fact, *Azolla* in the P-deprived, effluent fertilized tanks showed clear signs of P deficiency (photographic evidence in chapter 5.1.5).

Both *Azolla* and *Spirodela* produced least biomass in the tanks with added DAP although it was expected that addition of the allegedly limiting nutrient P would boost plant growth beyond that of effluent fertilized tanks. In the case of *Spirodela*, the tanks with added DAP showed rapid initial multiplication of *Spirodela* so that the surface was soon almost covered, allowing for little competition by algae. These almost pure *Spirodela* cultures yielded less biomass dry matter than those mixed with algae from the urine and effluent fertilized tanks. The difference in productivity also has to be put into perspective by the fact that variations between replications of the *Spirodela* / E + DAP treatment were higher than in other combinations. The reason for *Azolla*'s comparatively low biomass production in tanks with added DAP could not be identified; all *Azolla* tanks quickly reached full coverage and no algae were harvested along with *Azolla* biomass at the end. Other factors such as a different nutrient balance and/or spectrum of microorganisms might have played a role.

5.1.3 Annual Production

To make the obtained values comparable to previous research, biomass production has been converted to commonly used measures: Table 13 shows the average quantities of biomass (DM) produced by each treatment over the 22-day experimental period (per tank of 0.1735m^2), followed by values based on linear extrapolation to m^{-2}·d^{-1} as well as yearly production per ha which was found to be the most frequently used reference scale in the literature (note the different measuring units).

Table 13: Daily and annual biomass (DM) production in different growing media (linear extrapolation)

	average DM increase per tank in 22 days [g]	DM increase per $m^2 \cdot d^{-1}$ [g]	annual DM production per ha [t]
Azolla / Urine	10.69	2.80	10.22
Azolla / Effluent	11.68	3.06	11.17
Azolla / E+DAP	8.45	2.21	8.08
Spirodela / Urine	3.54	0.93	3.38
Spirodela / Effluent	3.01	0.79	2.88
Spirodela / E+DAP	2.60	0.68	2.49

Daily DM increase ranged roughly between 2 and 3g·m^{-2} for *Azolla* and below 1g·m^{-2} for *Spirodela*. Linear extrapolation of these values led to yearly DM quantities between 8.5 and 11.7t ha^{-1} for *Azolla* and between 2.5 and 3.4t·ha^{-1} for *Spirodela*.

DeBusk and Reddy (1987) observed *Azolla* production of 5.3g·m^{-2}·d^{-1} DM, almost doubling the quantities achieved during the experiment. For *Spirodela*, the literature presents very scattered productivity values: average annual yields between 10-30t·ha^{-1} DM seem to be realistic (Iqbal, 1999), but potential annual production of as much as 180t·ha^{-1} DM (near optimal conditions) and quantities as low as 2t·ha^{-1} DM (suboptimal conditions) have been recorded (Leng, 1999; Iqbal, 1999). Biomass productivity of *Spirodela* during the experiment was therefore in the lower range of the values stated in the literature. In any case, especially the highest mentioned productivity rates in the literature have to be treated with reservation: large-scale year-round field data is hard to come by and annual yields are often extrapolated from short-term lab experiments under controlled conditions. Such extrapolation can result in misleading data because in reality climatic conditions may halt primary production during part of the year and other factors (overcrowding, nutrient availability, possible diseases or infestation with pests, etc.) may change the growth patterns, making accurate and reliable predictions difficult.

One reason for the comparatively low recorded productivity during the experiment could be nutrient imbalances or deficiencies in the growing media. In natural water bodies with soil contact, minerals from the soil dissolve into the water and may serve as nutrients for the plants or at least influence the chemical composition of the water. During the experiment, nothing was added to the production tanks apart from well water, fertilizer (urine, effluent, DAP) and plant inoculum. In such an artificial “ecosystem,” a lack of micronutrients, microorganisms and their interactions might have restrained plant growth.

Other possible factors for slow growth include light incidence, too high or low pH, and the temperature profile in the Kathmandu Valley in early spring, which will be further discussed in chapter 5.4.

In addition, Leng (1999) describes a senescence and rejuvenation cycle which appears in both *Azolla* and duckweed species. The lifespan of fronds is definite; they can only produce a set number of daughter fronds after which they die. If the plants used for inoculation are from a single colony and all the same age, there will be a cyclical growth pattern with repercussions on short-term productivity. This phenomenon may cause considerable errors of interpretation in studies that examine, for example, the response of a few plants to differing nutrient sources over short time periods (ibid).

It also has to be remarked that the extrapolation leading to the annual yields in Table 13 was based on a linear growth curve which is very unlikely in the case of vegetative reproduction. Under favorable conditions, *Azolla* and *Spirodela* may follow a logistic growth model and go through a stage of exponential growth before the increase slows down and productivity reaches a plateau. Higher annual yields are therefore quite probable even with the moderate results obtained during the three-week-long field experiment. If an exponential growth phase is assumed ($K_t=K_o\cdot(1+p)^t$), the following daily growth rates and doubling times were established for the duration of the experiment (Table 14):

Table 14: Daily growth rates and doubling time of biomass in different growing media

	growth rate d^{-1} ($K_t=K_o\cdot(1+p)^t$)	doubling time [d]
Azolla / Urine	7.75%	9.29
Azolla / Effluent	8.20%	8.79
Azolla / E + DAP	6.82%	10.51
Spirodela / Urine	6.15%	11.61
Spirodela / Effluent	5.76%	12.38
Spirodela / E + DAP	5.18%	13.72

K_o: initial dry matter
K_t: final dry matter
t: duration of the experiment, 22 days
p: daily increase

The doubling times for *Azolla* were consistent with the range stated by Lumpkin (1983) who attests *A. caroliniana* 11 days doubling time at the beginning of spring and 2.9 days at the end of the season. *Spirodela*'s doubling time was much longer than the 24 hours mentioned for ideal conditions (cf. chapter 3.3.1) – indicating that the circumstances were suboptimal.

5.1.4 N-Content of *Spirodela*

The biomass analysis of *Spirodela* for its total N content yielded the following results (Table 15):

Table 15: Total N content of *Spirodela* in different growing media

treatment	total N content of Spirodela (% of dry matter)
Spirodela / Urine	0.62
Spirodela / Effluent	1.43
Spirodela / E + DAP	0.56

The N-content of *Spirodela* in the tanks fertilized with effluent more than doubled the values obtained for the other growing media.

These results suggest that *Spirodela* grown on diluted effluent concentrated N more in its tissue than the plants fertilized with urine and effluent plus DAP, respectively. The varying fractions of algae co-harvested with *Spirodela* from tanks with different growing media (cf. next chapter) may be partly responsible for the divergence, though the ranking of total N content does not match that of increasing algae content. However, the higher N content in the *Spirodela* / Effluent tanks corresponds to the higher NH_4-N removal observed in the same treatment.

The values for all three growing media are considerably lower than those stated in the literature. Gijzen (1997) reported an N-content of 4.80% of DM for wastewater-grown duckweed; Seidl et al. (2004) measured N-contents between 2 and 6% and registered a strong positive correlation with the N-concentration of the pond water: ~2.3% N-content in duckweed at 10mg·L^{-1} NH_4-N:, ~6% at 19mg·L^{-1} NH_4-N. Since the NH_4-N concentration in the experimental tanks fluctuated in the same range, it was surprising that the N-content of *Spirodela* turned out to be so much lower.

5.1.5 Observations of Plant Development

Graphic documentation of plant development and comparison between different growing media presented the following situation:

Azolla

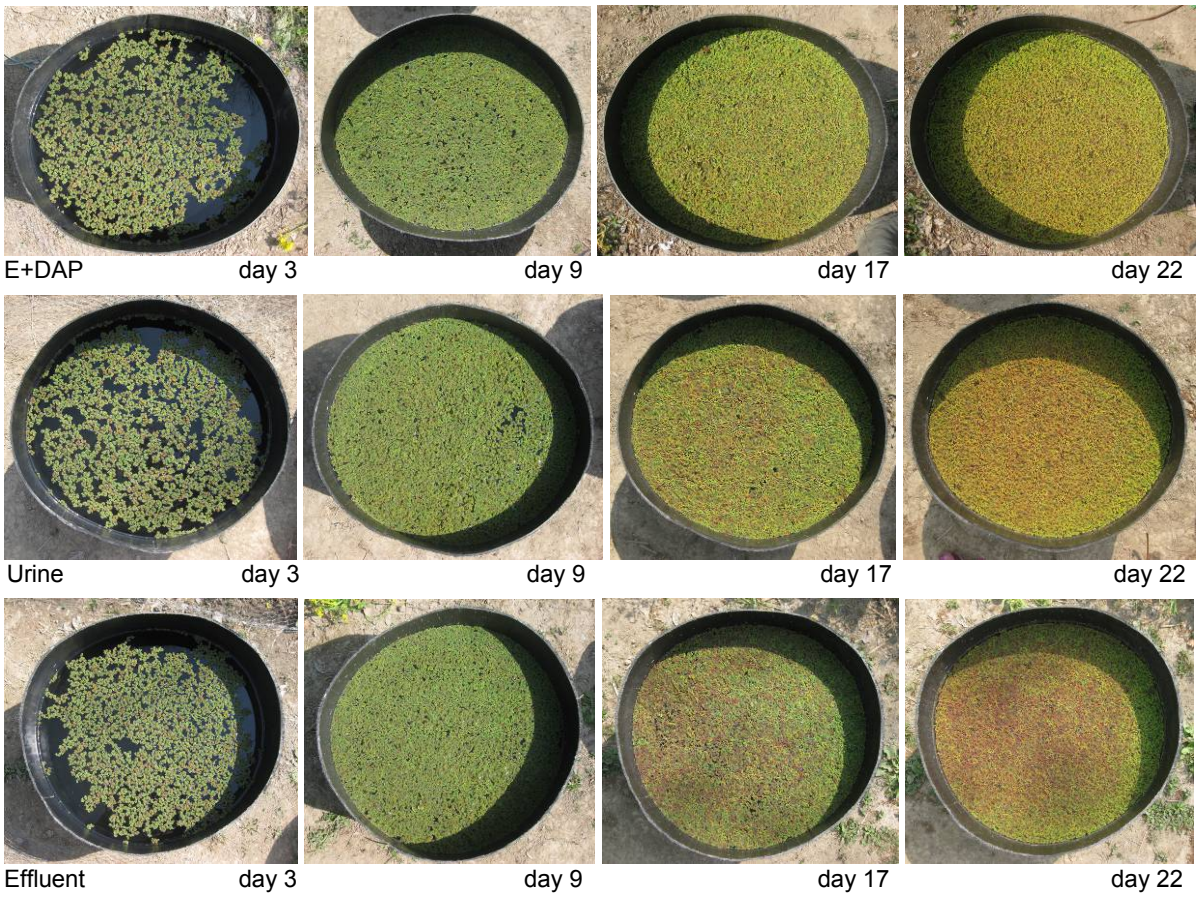


Fig. 29: *Azolla* development over time in different growing media

In all three growing media, *Azolla* reproduced quickly and covered the entire surface after the first week of the main experiment (Fig. 29). Thereafter, the plants started growing in multiple layers. Until the end of the experiment, *Azolla* in the tanks fertilized with urine gradually changed color from green to orange and the plants in the effluent fertilized tanks turned reddish-pink. *Azolla* in the tanks with effluent and added DAP also shifted towards a yellowish color compared to the initial green inoculum (close-up in Fig. 30). Plants in the edges near the tank rim stayed green for the most part in all growing media.

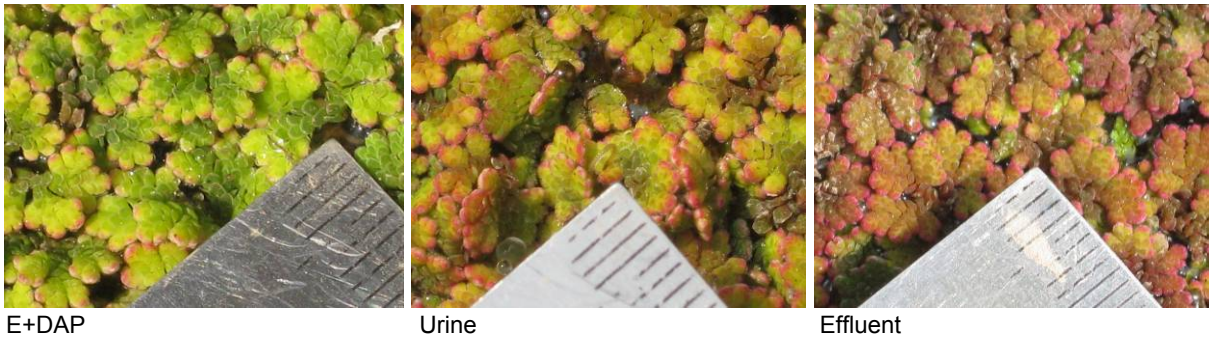


Fig. 30: Coloration of *Azolla* (close-up) in different growing media (day 22 of the experiment)

The orange to red color of *Azolla* in urine and effluent fertilized tanks was probably due to low P concentrations which are known to lead to such changes of appearance (cf. chapter 3.2.2). Since even the treatments with added P showed a slight change of color and *Azolla* on the often shaded edges remained green, it seems likely that exposure to sunlight also had an influence. This is supported by the fact that in ponds where *Azolla* occurs naturally, the top layer is usually red in color while those underneath show intensive green coloration.

Spirodela

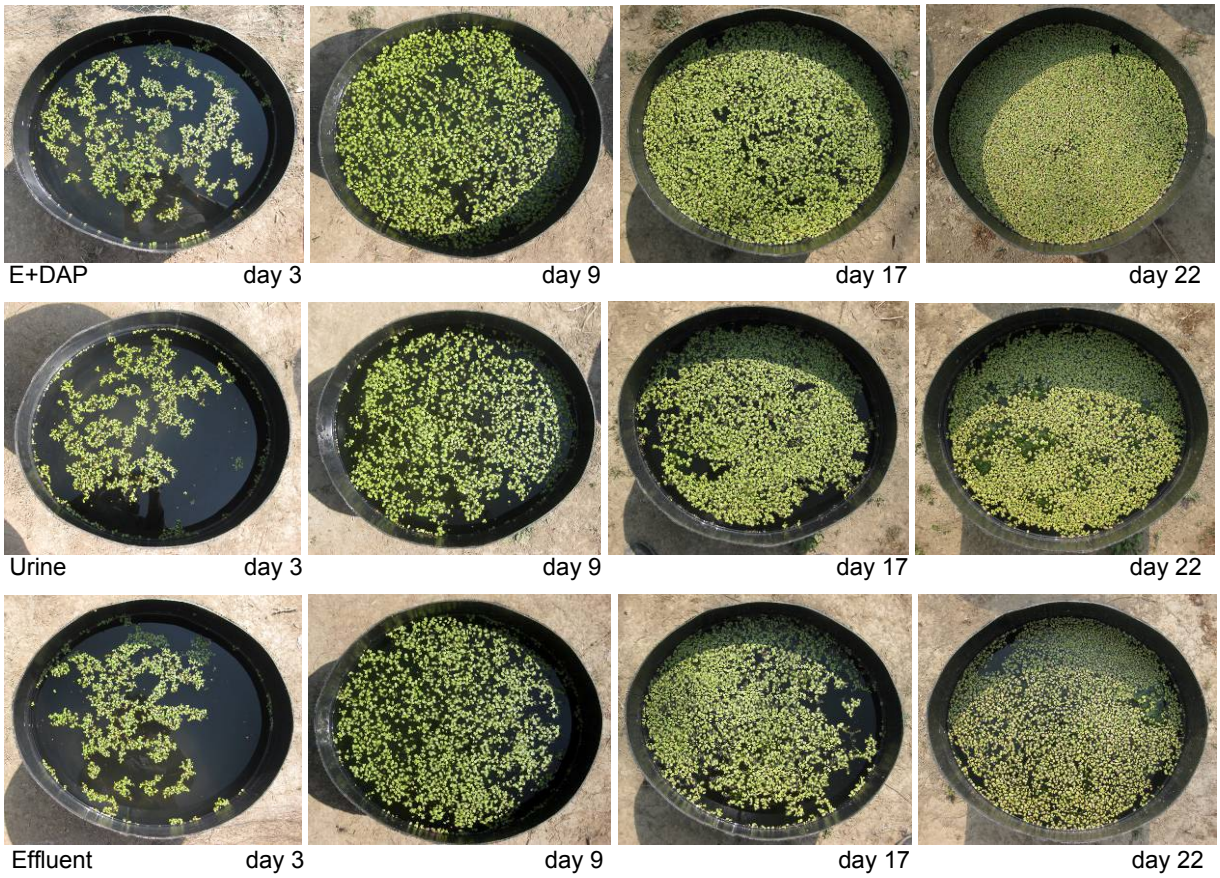


Fig. 31: *Spirodela* development over time in different growing media

In the tanks with effluent and DAP, *Spirodela* multiplied rapidly and covered the surface sooner than in the other tanks (Fig. 31). No algae could be observed in these tanks (E + DAP).

In urine fertilized tanks, green algae became visible after the first week of the experiment. They increasingly formed substantial aggregations suspended in the water and also appeared at the surface in the gaps between *Spirodela*. When the biomass was harvested at the end, algae accounted for a large portion of the recorded weight. The exact ratio could not be established because the algae were impossible to untangle from the *Spirodela* fronds and roots.

Spirodela in the tanks fertilized with effluent grew slowly and did not reach a dense cover until the end of the experiment. While some fronds turned brownish in all tanks, the number was noticeably higher in effluent tanks. Algae also came up, but at a later point and not to the same extent as in the urine tanks. Fig. 32 shows a close-up view of the plants in all three growing media.

E+DAP Urine Effluent

Fig. 32: *Spirodela* coverage and algae content in different growing media (day 22 of the experiment)

It is assumed that *Spirodela* in the tanks with effluent + DAP could grow vigorously thanks to sufficient P supply. Since the fronds covered the surface more quickly than in the other tanks, light penetration into the growing medium was lower and algae did not have a chance to come up. On the other hand, multiplication of *Spirodela* was slower in the urine fertilized tanks – possibly due to lower P concentrations – and the uncovered parts of the surface gave rise to algae growth. The same took place in the tanks with effluent, but the increase of algae was comparatively lower, which may be attributed to very limited availability of P.

According to Leng (1999), a certain number of brownish fronds is a natural phenomenon in *Spirodela* colonies both in laboratory and outdoor cultivation. They are dead mother fronds which have ended their activity after six deliveries of daughter fronds. The increased appearance of dead fronds in effluent fertilized tanks is thought to be an indication of slower rejuvenation rates and unsuitable growth conditions, i.e. probably a lack of nutrients (P) coupled with excessive solar radiation.

5.2 Nutrient Removal

5.2.1 Ammonium (NH_4-N)

Fig. 33 shows average NH_4-N removal over the 22-day experimental period for the six different treatments, both in absolute quantities [mg] and in percent of added NH_4-N:

	removed NH_4-N [mg]
Azolla / Urine	1267
Azolla / Effluent	1258
Azolla / E + DAP	1228
Spirodela / Urine	1316
Spirodela / Effluent	1536
Spirodela / E + DAP	1389

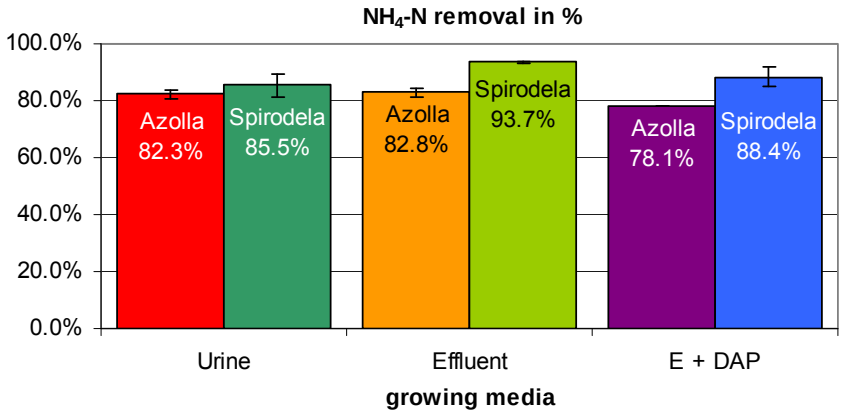


Fig. 33: NH_4-N removal in mg (left) and in % of added NH_4-N (right)

The highest NH_4-N removal rate was recorded for *Spirodela* / Effluent tanks with 93.7% (corresponding to 1536mg); the lowest for *Azolla* / E+DAP tanks with 78.1% (corresponding to 1228mg).

In all three growing media, *Spirodela* showed higher NH_4-N removal compared to *Azolla*. The difference between the two species is statistically very significant – or in other words, the probability (P) that the differences are only random and not associated with the different species is 0.12%.

Regarding the different growing media, *Azolla* showed almost equal NH_4-N removal (absolute quantities) from urine and effluent fertilized tanks and somewhat lower elimination from effluent + DAP tanks. For *Spirodela*, the NH_4-N removal from urine was clearly lower than from effluent fertilized tanks; removal from effluent + DAP lay in between. Statistically, the difference between growing media was significant for *Spirodela* (P = 4.17%) but not for *Azolla* (P = 43.72%).

Standard errors concerning removed NH_4-N were between 1 and 27mg for *Azolla* (E+DAP; Effluent, resp.) and between 5 and 63mg for *Spirodela* (Effluent; Urine, resp.). The differences between replications of the same treatment were highest for Spirodela / Urine. The standard addition performed for the photospectrometric NH_4-N analysis resulted in a standard deviation of 0.015 $mg \cdot L^{-1}$ NH_4-N for the calibration curve (method standard deviation) and 0.013 $mg \cdot L^{-1}$ NH_4-N for the retrieval function (cf. Appendix F).

Compared with the literature, NH_4-N removal rates around 90% seem within the usual range (Alaerts et al., 1996; Liu et al, 2008; Nhapi et al., 2003; Seidl et al., 2004). While Liu et al. achieved NH_4-N removal efficiencies >90% within five days under laboratory conditions, the study by Alaerts et al. reports reductions between 90-99% for a 20-day retention period of sewage in open-air duckweed lagoons in Bangladesh. The latter seems a better measure of comparison, although the dimensions of the experiments conducted in Nepal do of course not correspond to a large-scale system. The recorded removal rates are a few percentage points below those in Bangladesh, possible explanations being the suboptimal climatic conditions during the experiment, a potential lack of micronutrients restraining plant growth, and low initial plant coverage (denser populations may reduce nutrients more efficiently).

The lower removal rates in *Azolla* vs. *Spirodela* tanks could be an indication that *Azolla* not only removed N from the growing media but met part of its N needs through N_2 assimilation from the atmosphere. The comparatively low NH_4-N removal in the *Azolla* / E+DAP tanks was contrary to expectations; it was assumed that additional P would also increase N uptake rates thanks to faster development of biomass (which was not the case, either; cf. chapter 5.1 above).

Contribution of Spirodela to Total NH_4-N Removal

Based on the total N content of *Spirodela* determined at KU (chapter 5.1.4), biomass production and NH_4-N removal from production tanks, Table 16 below shows what percentage of removed N was actually assimilated by the plants:

Table 16: Proportion of NH_4-N assimilated by *Spirodela*

treatment	fraction of NH_4-N assimilated by *Spirodela*
Spirodela / Urine	1.67%
Spirodela / Effluent	2.80%
Spirodela / E + DAP	1.05%

The contribution of *Spirodela* towards NH_4-N removal was highest in effluent fertilized tanks and lowest in those with E+DAP. To put the results in perspective, Table 17 presents comparative data.

Table 17: N removal through duckweed in the literature

authors	fraction of N removal attributable to duckweed
Alaerts et al. (1996)	~42%
Körner and Vermaat (1998)	30-47%
Iqbal (1999)	50% (+/- 20%)
Seidl et al. (2004)	almost 30%

The calculated ratios of NH_4-N assimilated by *Spirodela* are clearly far below those stated in the literature. The cited authors all mention duckweed to be responsible for 30-50% of total N-loss by uptake of NH_4^+. The remaining decrease is attributed to other processes that influence the N balance in duckweed ponds: nitrification/denitrification, volatilization of NH_3, microbial uptake and sedimentation (Alaerts et al., 1996, Iqbal, 1999; Seidl et al, 2004). Especially at alkaline pHs above 8, NH_3 volatilization plays a major role (Iqbal, 1999). pH measurements during the experiment were not sufficient to indicate a tendency towards alkaline conditions and possibly increased volatilization (see chapter 5.4.3). It must be concluded that N uptake by plants (≤ 2.8%) was minimal compared to N loss (≥ 97.2%) through other processes.

Factors that led to the low share of *Spirodela* in total NH_4-N removal include its low biomass production, low dry matter content, and low total N content, all of which have been discussed above. Some of the measurements and analyses might have produced erroneous results, and mistakes might have magnified if faulty values were used for the final calculation (further sources of error cf. chapter 5.5).

5.2.2 Phosphate (PO_4-P)

Fig. 34 shows average PO_4-P removal over the 22-day experimental period for the six different treatments, both in absolute quantities [mg] and in percent of added PO_4-P:

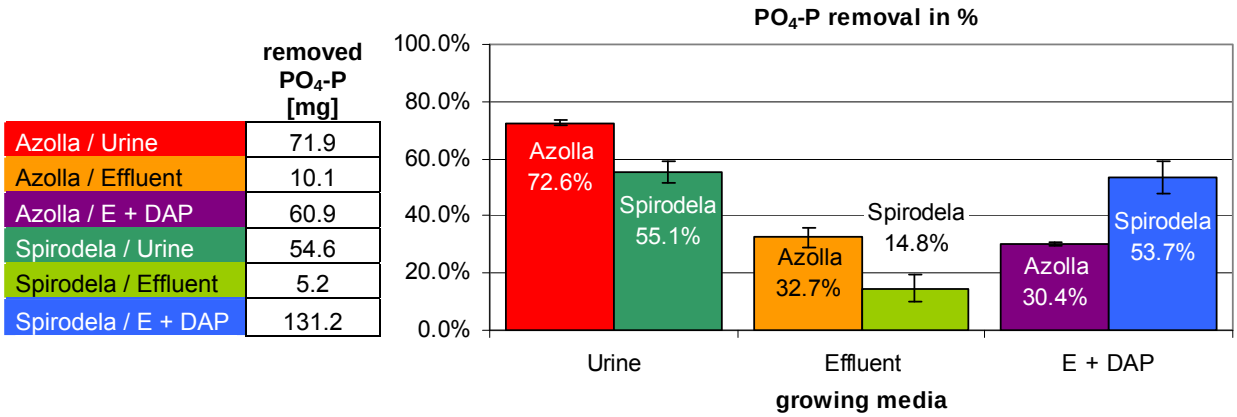


Fig. 34: PO_4-P removal in mg (left) and in % of added PO_4-P (right)

The highest PO_4-P removal rate was recorded for *Azolla* / Urine tanks with 72.6% (corresponding to 71.9mg). Calculations produced the lowest removal rate for *Spirodela* / Effluent tanks with 14.8% (corresponding to 5.2mg). *Azolla* had higher PO_4-P removal rates than *Spirodela* in urine and effluent; *Spirodela* was more efficient in E + DAP fertilized tanks where absolute PO_4-P removal in mg was highest across all treatments (131.2mg) (Fig. 34). Overall, the difference between *Azolla* and *Spirodela* in terms of absolute PO_4-P removal [mg] is not statistically significant, i.e. there is low probability that PO_4-P removal is dependent on plant species (P = 45.57%). However, absolute PO_4-P removal [mg] is very significantly related to the different growing media for both *Azolla* and *Spirodela* (P = $5.54 \cdot 10^{-6}$% and 0.012%, resp.), i.e. the growing media were a decisive factor in the PO_4-P removal the plants achieved.

Standard errors were between 1 and 4mg, apart from *Spirodela* / E+DAP tanks where it amounted to 14mg. A standard addition performed by Etter (2009) for the same analysis method and photospectrometer resulted in a standard deviation of 0.098mg·L^{-1} PO_4-P.

Values for P removal in the literature vary greatly: While Costa et al. (1999) state about 36% P removal efficiency, Nhapi et al. (2003) achieved removal rates of 50%, and Seidl et al. (2004) even reached 80% P removal efficiency. In this context, the PO_4-P removal rates calculated for urine attest particularly *Azolla* a good performance, whereas those for effluent are below the rates stated in the literature. As Rejmankova (1982) pointed out, though, the plants cannot substantially remove P anymore at concentrations below 4mg·L^{-1} – which was the case in urine as well as effluent tanks (cf. chapter 3.3.2).

In addition, especially the low PO_4-P removal rates calculated for effluent need to be treated with suspicion: Some of the weekly measurements of growing media samples during the experiment surprisingly produced negative removal results, i.e. the PO_4-P load turned out higher than what had been in the tanks the week before (fertilizer addition included). These unexpected values are highlighted bright green in the nutrient removal result sheets in Appendix E. The following are possible explanations for apparent P accumulation in some of the tanks during periods of the experiment:

- Errors of analysis, either of the growing media samples or of the urine, effluent, and DAP used for fertilization. If the actual PO_4-P content of fertilizers was higher than its measured concentration, more PO_4-P than recorded was added to the tanks, potentially resulting in higher measured concentrations in growing media samples the following week.
- PO_4-P inputs from a source other than analyzed and calculated PO_4-P loads applied through fertilizers, e.g. because (1) parts of total P from the water or fertilizer turned into ortho-PO_4-P, (2) the well water contained higher or varying levels of P than determined in the initial analysis → added water to compensate for evaporation increased P content in the tanks, (3) decomposing plants released nutrients back into the tanks. The latter phenomenon has been described by Leng (1999) regarding duckweed whose stored P becomes rapidly available in a highly soluble form as soon as the plant is disrupted or dies. In the effluent fertilized duckweed tanks this is likely to have happened as the proportion of dead fronds appeared higher than in other treatments (cf. chapter 5.1.5).

5.2.3 Potassium (K)

	removed K [mg]
Azolla / Urine	38
Azolla / Effluent	152
Azolla / E + DAP	130
Spirodela / Urine	-112
Spirodela / Effluent	37
Spirodela / E + DAP	18

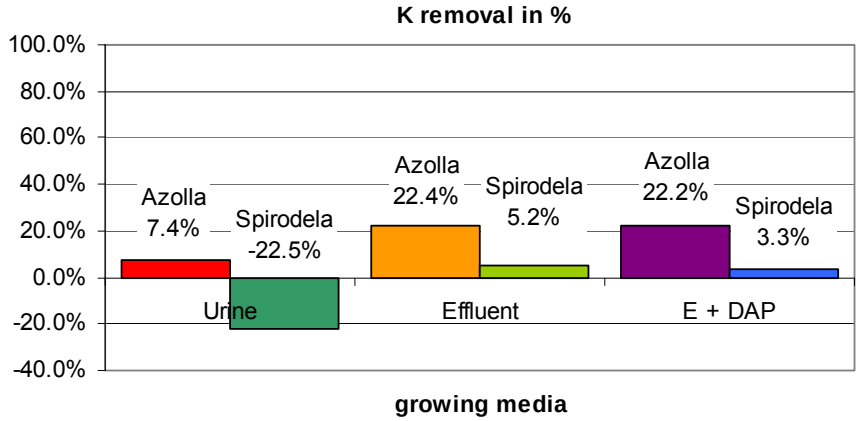


Fig. 35: K removal in mg (left) and in % of added K (right)

Fig. 35 shows average K removal over the 22-day experimental period for the six different treatments, both in absolute quantities [mg] and in percent of added K.

Highest K removal rates were achieved in *Azolla* / Effluent tanks with 22.4% (corresponding to 152.1mg); lowest K removal was recorded for *Spirodela* / E+DAP tanks with 3.3% (corresponding to 18.2mg). *Spirodela* / Urine tanks showed a positive K balance, i.e. no removal but an increase of 22.5% (112.1mg) K was calculated, based on the results from weekly fertilizer and growing medium sample analyses (cf. result sheets for K, Appendix E). *Azolla* showed higher K removal rates than *Spirodela* in effluent and E + DAP tanks; its removal was also higher in urine tanks in view of the K accumulation in *Spirodela* tanks of the same growing medium (Fig. 35).

Both *Azolla* and *Spirodela* achieved their highest K removal in effluent fertilized tanks, followed by E + DAP, and lowest K removal from urine fertilized tanks.

K removal rates were considerably lower than those of NH_4-N and PO_4-P, which suggests that K is less readily taken up by the plants than N and P and possibly also less of a limiting factor for them.

The recorded removal rates have to be looked at carefully, though: They do not represent an average of three replications but are only based on sample analyses from a single tank per treatment. Moreover, the increase of K in the *Spirodela* / Urine treatment as well as in other tanks in the course of the experiment ask for a critical examination of possible causes that led to such results. The negative removal values are highlighted bright green in the result sheets in Appendix E.

As for PO_4-P, reasons for the increase might be higher K content of the well water than initially measured or the release of K into the water from decaying plants. Also, measured K concentrations for urine and effluent raised a question as there was more K in the effluent than in the urine from which it had been produced. This could be due to contaminated $MgSO_4$ which might have added additional K to the effluent during struvite production. Another explanation could be the removal of precipitated K from urine and effluent samples through filtration before analysis at Eawag in Switzerland: since the samples were acidified in Nepal and analyzed only a few weeks later, they were filtered again because floating compounds had formed in the meantime. If the analyzed concentration of K in urine was lower than the quantities added to the tanks, the calculations based on sample analysis do not reflect the K conditions in the tanks during the experiment.

To conclude, further studies will have to be conducted to determine K removal rates more reliably.

5.2.4 Correlations Nutrient Removal – Biomass Production

NH_4-N Removal – Biomass Production

Statistical evaluation revealed no significant correlation between NH_4-N removal and biomass production for either species. The determined correlation values were r = 0.470 for *Azolla* and r = -0.267 for *Spirodela*; both lying below the critical value of [r] > 0.67 (sample size 9). Fig. 36 shows the distribution of the different treatments graphically.

Azolla generally shows lower NH_4-N removal and higher biomass production; *Spirodela* higher NH_4-N removal and lower biomass production. No clear trend of a relationship between NH_4-N removal and biomass production is visible.

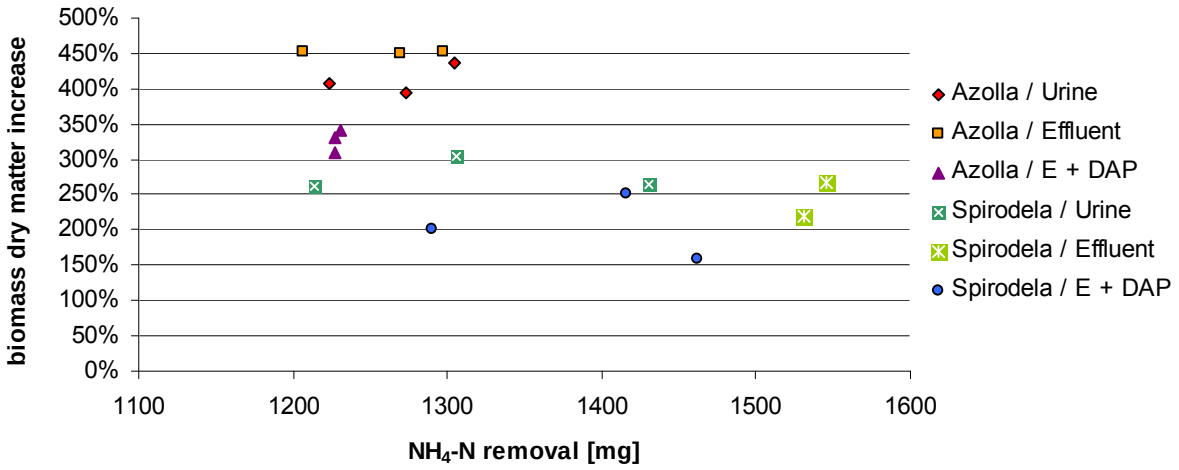


Fig. 36: Relationship between NH_4-N removal and biomass production

An explanation for the absence of an apparent connection between the two parameters could be that the bulk of NH_4-N removal is not attributable to assimilation by plants, but to other factors such as volatilization of NH_3 that are more or less independent of biomass production. ("Removal" stands for the sum of plant uptake and loss.) Since only few uptake values – based total N analysis of *Spirodela* biomass samples – were available and loss through other processes could not be determined, it is difficult to draw conclusions.

PO_4-P Removal – Biomass Production

As for NH_4-N, statistical evaluation shows no significant correlation between PO_4-P removal and biomass production for either species. The correlation values were r = -0.556 for *Azolla* and r = -0.305 for *Spirodela*; both lying below the critical value of [r] > 0.67 (sample size 9). Fig. 37 shows the distribution of the different treatments graphically.

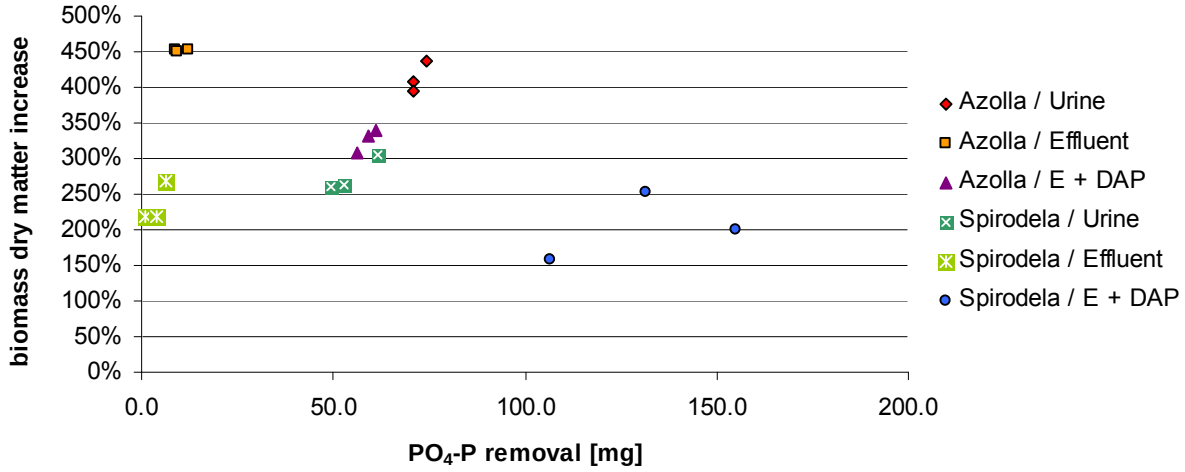


Fig. 37: Relationship between PO_4-P removal and biomass production

If E + DAP fertilized tanks are left out, A*zolla* shows higher biomass production and slightly higher PO_4-P removal than S*pirodela*, though there is again no clear indication of a relation between PO_4-P removal and biomass production. For *Spirodela*, the level of biomass production is relatively constant, independent of PO_4-P removal.

It is noticeable that even with added DAP, biomass production did not increase; on the contrary, average growth was lower than for *Spirodela* in urine and effluent. This is all the more surprising as P was expected to be the main limiting factor for biomass production. It could be, however, that other factors such as micronutrients or pH conditions had an even stronger limiting effect so that P availability became of secondary importance. These parameters were not thoroughly checked during the experiment and should be further investigated.

K removal – Biomass Production

The statistical evaluation revealed no significant correlation between K removal and biomass production either. The correlation values were r = 0.199 for *Azolla* and r = -0.738 for *Spirodela*; both lying below the critical value of [r] = 1 (sample size 3). Fig. 38 shows the distribution of the different treatments graphically.

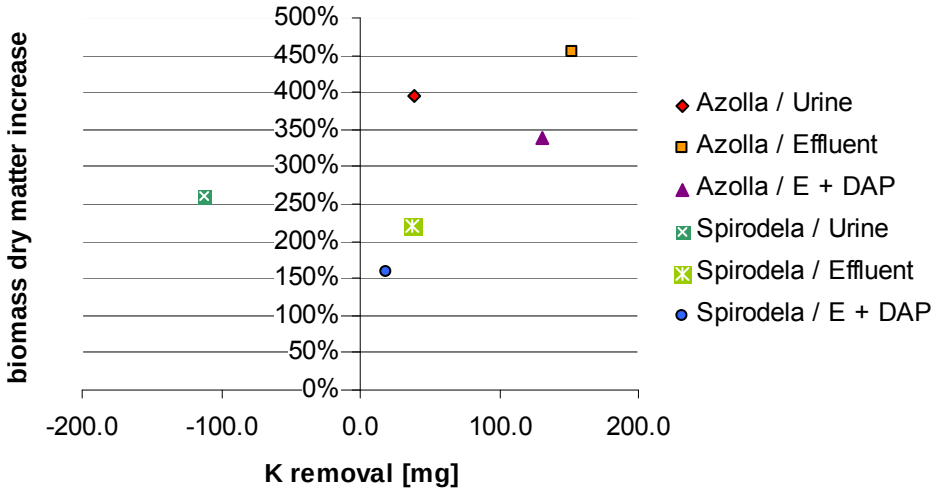


Fig. 38: Relationship between K removal and biomass production

Azolla shows higher biomass production and higher K removal; *Spirodela* lower biomass production and lower K removal, but within the species there is no clear trend for a correlation between biomass production and K removal. The increase of K in the *Spirodela* / Urine treatment as well as the low sample size – only three samples per plant species – make substantive interpretation of the obtained data impossible.

5.3 Health Issues – Further Use of Biomass

The well water analysis revealed the following profile of micronutrients and heavy metals (first row in Table 18):

Table 18: Micronutrient and heavy metal concentrations of well water

	Na	Mg	Al	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd
average concentration in well water (J. Maharjan's farm) [mg·L^{-1}]	6.2375	4.6505	0.0727	0.4209	0.0011	0.0178	0.0647	0.0001	0.0011	0.0023	0.0533	0.0019	-0.0003
WHO guideline value (WHO, 2003) [mg·L^{-1}]	-	no mention	-	no mention	0.05	0.4	-	no mention	0.07	2	-	0.01	0.003
typical concentrations in natural water bodies (WHO, 2003) [mg·L^{-1}]	<20	no mention	no mention	no mention	<0.002 (drinking water)	0.001-0.2	0.5-50	no mention	<0.02	≤0.005	≤0.05 (ground-water)	0.001-0.002	<0.001 (drinking water)

The concentration for Cd was below the limit of determination of 0.1 ppb (0.0001mg·L^{-1}) so that the value turned out negative.

The WHO has issued guideline values for water regarding the concentration of elements with potentially detrimental effects on human health (second row in Table 18). Based on the average daily water consumption and food intake, these values assure that the tolerable upper intake level is not exceeded (WHO, 2003). Some of the tested elements are not listed in the WHO guidelines ("no mention"), others are discussed but no health-based guideline value is proposed ("-") because they normally occur in concentrations far below those at which toxic effects may take place. The WHO further cites typical concentrations in natural water bodies for a number of elements (third row in Table 18).

A comparison with the well water used in the experiment showed that none of the tested elements exceeded the guideline values and all of them were within the typical concentration range. It can therefore be assumed that the well water is suitable for aquaculture and does not pose an elevated risk in terms of heavy metal accumulation. The question whether micronutrients were available in sufficient quantities or possibly caused deficiencies in *Azolla* and *Spirodela* lay beyond the scope of this thesis and would have to be investigated in further studies.

The only other potential source of heavy metals could be the fertilizers (urine and its effluent) added to the production tanks. However, heavy metal concentrations in urine are reported to be low compared with other organic fertilizers, and even though Cu, Hg, Ni, and Zn were found to be higher than in precipitation and surface waters (Kirchmann and Pettersson, 1995), the 125-145fold dilution in the tanks diminishes the risk of excessive accumulation.

As to bacterial contamination, a single urine sample analyzed by ENPHO in Nov. 2009 for *E. coli* (Lab. Reg. No. 732) showed a count of zero CFU per 100ml. Although a positive outcome, this is not a conclusive result and more research about microbiological contamination is in progress at Kathmandu University.

5.4 Environmental Parameters

5.4.1 Temperature: Seasonal Variations

As can be seen in Fig. 39 below, both ambient air temperature and average water temperature in the experimental tanks increased over the duration of the experiments: from 10.1 and 8.2°C respectively in late January to 20.5 and 15.2°C in mid March (for raw data of measurements see Table 32 and Table 33, Appendix G). The measurements were interpolated between the data points. Ambient air temperature showed more pronounced fluctuations than water temperature. The temperature differences between the experimental tanks resulted in standard deviations between 0.12 and 0.71°C.

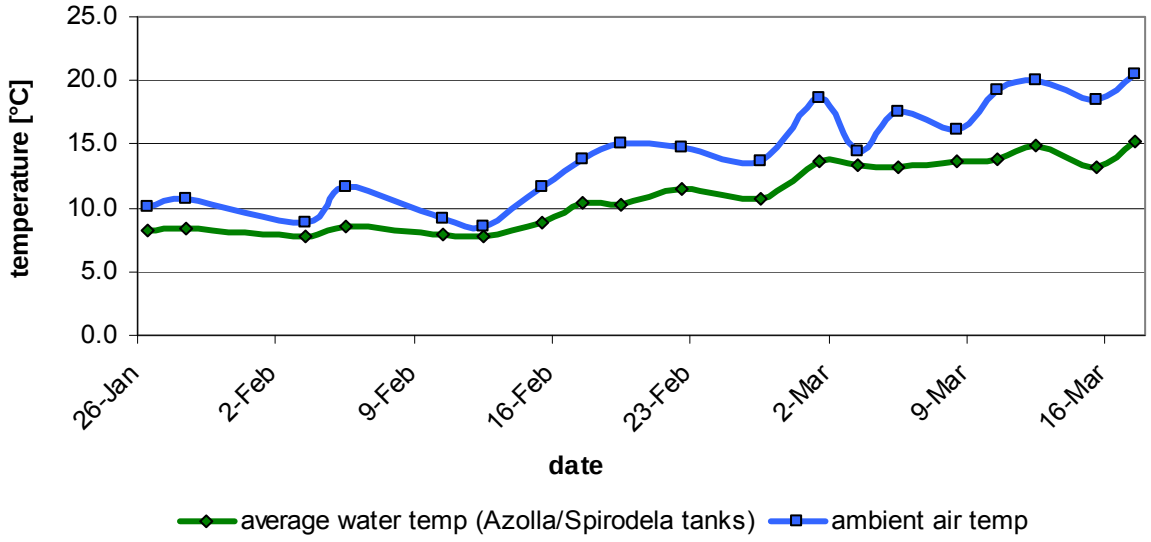


Fig. 39: Seasonal temperature variations (January – March 2010)

The rising temperature in the course of the experiment was a positive development with regard to plant requirements – but even in March it lay below the optimum as stated in chapters 3.2.1 and 3.3.1. It has to be noted, however, that measurements were taken in the morning and that water temperature in the tanks increased by over 10°C until late afternoon on sunny days (cf. Fig. 41).

The temperature difference between the tanks was negligible and obviously attributable to the time lag between measurements from the first to the last tank. On clear and sunny days, the temperature increased rapidly in the morning hours so that the approximately 30-minute gap between measurements in the first and the last tank was responsible for much of the difference. Varying coverage ratios may also have played a minor role as suggested by the 24-hour temperature measurement (cf. following chapter).

Temperature-wise, the summer half-year starting from April would be more suitable for *Azolla* and *Spirodela* cultivation in Kathmandu than winter and early spring, as has been confirmed by the Botanical Gardens and the Nepal Agricultural Research Council (Thapa, 2009; Khadka, 2009). Between December and February, the average air temperature reaches values below 5°C (left diagram in Fig. 40), and while both *A. caroliniana* and *S. polyrrhiza* should be able to survive, growth is likely to be minimal during those periods.

The Terai is expected to be better suited for cultivation even during the winter months (cf. temperature diagram for the city of Bhairahawa, Fig. 40), but the hot and dry spring season before the onset of the monsoon might raise temperatures in shallow ponds close to or even beyond the upper tolerable limit for *Azolla* and *Spirodela*.

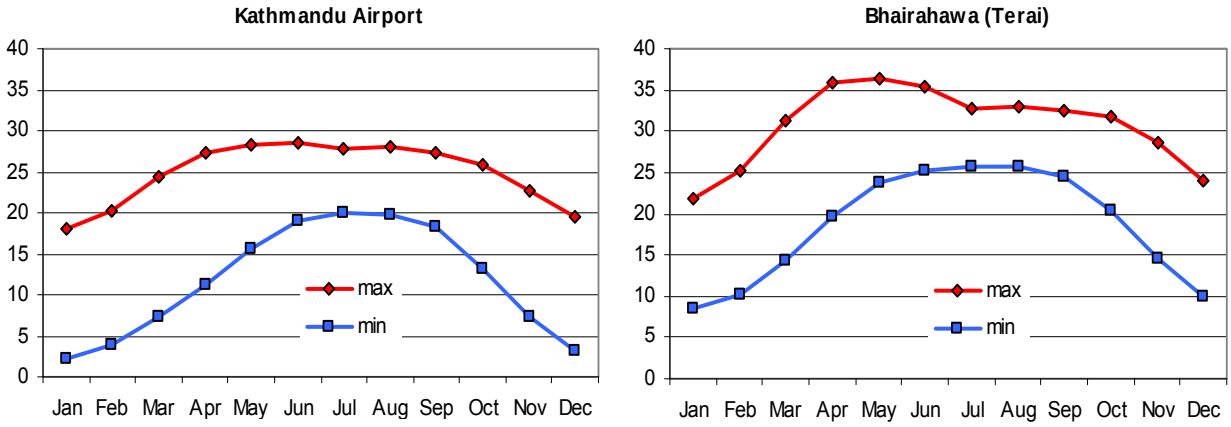


Fig. 40: Average maximum and minimum temperatures [°C] of Kathmandu Airport (left) and Bhairahawa (right) (based on data from the Government of Nepal, 2006) **Temperature: Diurnal Fluctuation**

The 24-hour measurement (Fig. 41) showed that ambient air temperature fluctuated between 6.1°C in the early morning before sunrise and 25.6°C in the early afternoon, thus comprising a temperature span (ΔT) of 19.5°C (for raw data of measurements see Table 34, Appendix G). The data was interpolated between the hourly measuring intervals.

In the experimental tanks the temperature fluctuation was staggered by two hours in the afternoon and by one hour in the early morning, reaching an average maximum of 26.8°C and an average minimum of 10.8°C. The temperature span (ΔT) amounted to 16°C; 3.5°C lower than that of ambient air temperature. In comparison, the fluctuation in the unplanted evaporation control tank was slightly greater.

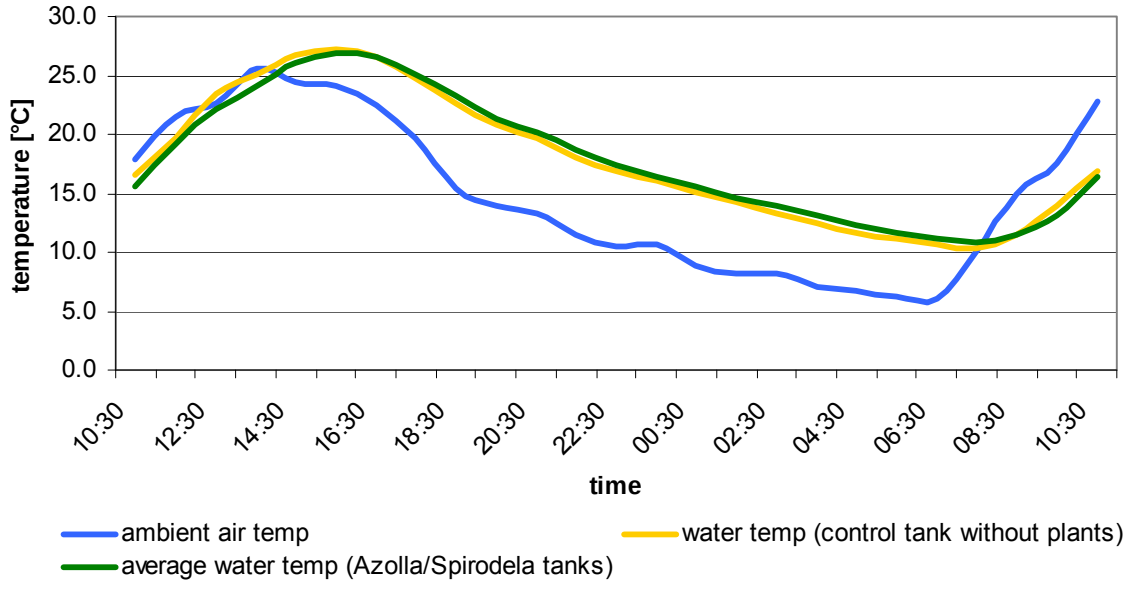


Fig. 41: Diurnal temperature fluctuations (March 4/5, 2010)

The measurements were in accordance with the thermodynamic principle that temperature fluctuations in water (i.e. in the experimental tanks) are smaller than in air thanks to the higher specific heat capacity

of water. However, due to the small volume contained in the tanks, the fluctuations were still considerable and might potentially have had a negative impact on the plants whose optimum temperature range lies clearly above the night time temperatures measured in March (cf. plant requirements, chapters 3.2.2 and 3.3.2). In places where *Azollaceae* and *Lemnoideae* are used on a larger scale for nutrient recovery from wastewater (e.g. duckweed cultivation in Bangladesh, UNEP/GPA et al., 2004; Iqbal, 1999), the ponds comprise a much larger volume and are therefore less susceptible to diurnal temperature fluctuations.

Plant coverage ratio also had a slight influence on temperature fluctuations as the values obtained for the unplanted evaporation control tank showed. *Spirodela* might therefore have been at a disadvantage against *Azolla* due to lower inoculation density that left much of the surface uncovered.

5.4.3 pH

During the main experiment, pH monitoring only covered three measurements during the first week due to a defect display. The recorded values ranged between 7.90 and 8.56; they were generally higher for *Spirodela* than *Azolla* and increased towards the end of the week (Fig. 42).

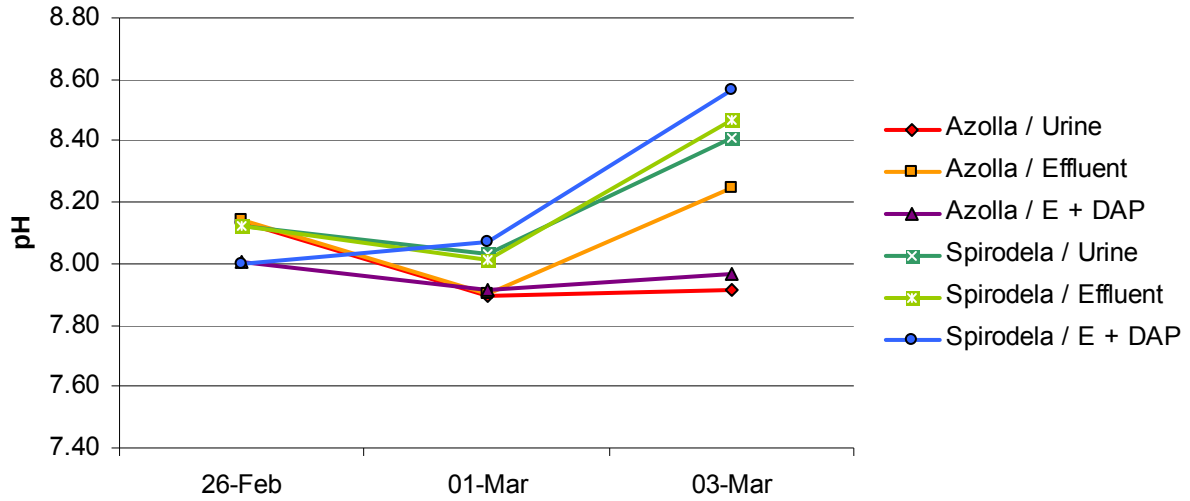


Fig. 42: Average pH levels during the first week of the experiment

The measured pH lies above the optimal range for both plant species (*Azolla*: 4.5-7; *Spirodela*: 6.5-7.5); even more so as the measurements were taken in the morning and pH generally rises during the day when the plants take up CO_2 from the water for photosynthesis. The tolerance limits were not exceeded, though, at least not during the three morning measurements. Since further monitoring over the entire period of the experiment and especially the 24-hour measurement for diurnal fluctuations could not be carried out, the collected data is insufficient for a reliable interpretation.

One assumption that can be made nevertheless is that *Spirodela* tanks probably showed higher pH values than those with *Azolla* due to lower biomass and therefore less respiration at night. Respiration leads to higher CO_2 concentrations and thus lowers the pH of the growing medium.

5.5 Assessment of Methodology

5.5.1 Experimental Set-up and Procedures

Limited Number of Replications

Working with only three replications per treatment (18 tanks altogether) led to restricted validity of the obtained results. Extreme values influenced the average, and especially some of the values for nutrient removal were widely strewn which made comparison between different treatments somewhat arbitrary. For K removal, only samples from a single tank per treatment were analyzed, ruling out any cross-check of the correctness of the data.

A larger number of replications would doubtlessly be an advantage, produce a larger picture and more reliable results. For the conducted experiments the number of replications had to be kept low due to limited capacity to manage and monitor the tanks as well as analyze the samples.

Seasonal Timing

The timing of the experiments in the winter and early spring months was not optimal because of low temperatures. Nepali experts (Khadka, 2009; Thapa, 2009) advised against trials with plants sensitive to low temperatures (spirulina) since the required range could not be provided between January and March. In the summer half year, the cultivation of spirulina might have been possible – though even successful production would not have solved the difficulty of keeping the culture alive over the winter. Khadka and Thapa also pointed out that it might be difficult to find inoculum of the chosen species (*Azolla* and duckweed) in January, which actually turned out to be the case for *Spirodela*.

It was, however, not necessarily a drawback to conduct the experiments from January to March: although temperatures were below the optimum for both *Azolla* and *Spirodela*, the research could provide information on the feasibility of cultivation under the given seasonal conditions, which may be useful if year-round production of these plants is striven for.

Low Inoculation Density, Competition from Algae

Azolla was abundantly available and could be used in inoculation quantities (60g) tripling those of *Spirodela* (22g). Less initial plant material led to a disadvantage of *Spirodela*: when light penetrates into the water due to incomplete plant coverage, algae growth is stimulated and leads to competition for oxygen and nutrients. In case of algal blooms, the pH may rise and lead to a loss of nutrients (e.g. NH_3 volatilization) (Lumpkin, 1987).

Competition itself is not necessarily a problem in terms of nutrient removal as long as algae growth is moderate and nutrients are taken up by plants (including algae) – only the intended use of the biomass will be affected. Algae are more difficult to harvest than floating plants like *Azolla* and *Spirodela*, but their use for biogas production is promising (cf. chapter 2.4.2).

If *Spirodela* is the desired crop, higher inoculation densities are required. Further literature research showed that an initial coverage ratio of half the surface area (Leng, 1999) or at least 70g fresh material per tank ($400g \cdot m^{-2}$; Rodriguez and Preston, 1996; Hamdi, 1982) would have been advisable.

Biomass Weighing

It would be interesting to follow biomass increase on a more frequent (e.g. weekly) basis – but a more plant-friendly extracting and weighing procedure would have to be developed because the method used in the experiment led to plant damage during the preliminary phase. Van Hove et al. (1987) describe a device that allows periodical weighing of *Azolla* with minimal disturbance of the population: a mosquito net replacing the bottom of production tanks and also permitting standardized drainage for consistent results.

5.5.2 Laboratory Analyses

The following are possible sources of error that may have influenced the outcome of the results:

Photospectrometric Nutrient Analyses

Imprecision is likely to have happened during the measurements, especially while analyzing NH_4-N content of urine and effluent where two-step dilution was necessary. The results may also have been affected by cross-contamination between samples due to insufficiently cleaned glassware and/or use of contaminated tap water. The quality of the distilled water and of its later replacement (bottled drinking water) could not be ascertained either. If faulty results were used for calculating the required fertilizer quantities (urine, effluent, DAP), all subsequent calculations (nutrient removal) will have been affected. This could have been the reason for the negative removal rates mentioned in chapters 5.2.2 and 5.2.3. Nevertheless, the standard additions showed relatively low standard deviations of 0.013 $mg \cdot L^{-1}$ for NH_4-N and 0.098$mg \cdot L^{-1}$ for PO_4-P.

Determination of Dry Matter and Total N

The methods used for establishing the dry matter conversion factor (12 hour drying at 105°C) and for total N analysis of duckweed (Icarda, 2010) may have been unsuitable, producing results that were lower than the actual dry matter and N content of the plants. This could have led to the very low proportions of N assimilated by duckweed, which are not supported by the literature. It has to be noted, though, that biomass samples did not consist of pure duckweed but also included algae, so that comparison with duckweed values from previous research might be questionable from the start.

Moreover, the biomass drying and total N analysis comprised only one biomass sample per treatment, mixed from the three replications in equal proportions. Accuracy of the obtained results could therefore not be cross-checked.

Calculations

The method used for calculating nutrient removal might not have been appropriate. For instance, it did not account for total P and small concentrations of NO_3 in the well water, urine and effluent. These nutrients might have been converted into plant available ortho-PO_4^{3-} and NH_4 (through nitrification) in the course of the experiment, or taken up by the plants directly (NO_3).

6. Conclusions and Further Research Needs

The field experiments yielded the following findings regarding biomass production and nutrient removal by *Azolla caroliniana* and *Spirodela polyrrhiza*, grown on diluted urine and struvite production effluent as well as a control treatment with added DAP:

Performance of Plant Species

Azolla produced more biomass than *Spirodela* in all growing media (dry matter increase between 327 and 452% vs. 204-277%). Better results for *Spirodela* might be obtained with higher inoculation density (> 400g·m^{-2}) and in shaded ponds – but inoculum is scarce around Kathmandu in the winter months.

Regarding nutrient removal, PO_4-P and K were removed more efficiently from *Azolla* tanks while *Spirodela* tanks achieved higher removal rates for NH_4-N (as much as 93.7% removal within 22 days). Only a small percentage of N (2.8%) was actually assimilated into the plants, though. Most N was probably lost through denitrification and NH_3 volatilization.

Suitability of the Growing Media

Urine as a growing medium led to higher biomass production for *Spirodela*; effluent for *Azolla.* However, P deficiency already became apparent in effluent fertilized *Azolla* plants (red coloration) and is very likely to inhibit growth in the long run. Added DAP did not increase biomass quantities for either species, but the plants looked healthier and the risk of competition from algae was reduced.

NH_4-N removal was almost equal from urine and effluent by *Azolla* (82.3 and 82.8%, respectively); *Spirodela* achieved higher removal rates from effluent (93.7%) than urine (85.5%). PO_4-P removal efficiency was higher from urine than from effluent for both species (73% by *Azolla* and 55% by *Spirodela*) possibly because the level of P in effluent fertilized tanks was too low. K removal was higher from effluent than from urine for both species, but the removal efficiency was generally low (between 5.2 and 22.4%); *Spirodela* / Urine tanks even showed an increase of K in the course of the experiment, possibly due to inappropriate sampling, analysis, or calculations.

Correlations

There was no significant correlation for either of the species between removal of nutrients (NH_4-N, PO_4-P, K) and biomass production. In the case of NH_4-N, nutrient removal is likely to be dominated by processes independent of plant uptake.

Health Issues – Heavy Metals, Pathogens

The well water used in Siddhipur creates no increased risk of heavy metal accumulation; all tested metals were below critical concentrations according to WHO guidelines for drinking water. This result only relates to the used water source and is not valid for other places.

Although *E. coli* was not present in an analyzed urine sample, the possible transfer of pathogens through urine / effluent fertilized aquaculture requires more studies.

Temperature, pH

From late February onwards the temperature in the Kathmandu Valley can be considered suitable for the cultivation of *Azolla* and *Spirodela* although it still drops considerably at night. Earlier in the year, plant growth is likely to be restrained by low temperatures both during the day and especially at night.

pH monitoring was not sufficient to determine whether or not the conditions were favorable.

Qualification of Results

The stated results have to be treated with reservation as the conditions under which they were obtained do not correspond to research carried out by well-equipped professionals. Inexperience of the author, the choice of methods, and imprecision or cross-contamination during chemical analyses are likely sources of error affecting the outcome of the experiment itself as well as biomass and nutrient removal calculations. To confirm the findings, more and longer carefully planned field trials are necessary.

Summary of Key Findings

Aquaculture with *Azolla* and *Spirodela* for nutrient removal from urine under the climatic conditions of early spring in Kathmandu is possible. Effluent as a growing medium can only be recommended for short term treatment: Even though in some treatments better nutrient removal and/or biomass production rates were recorded over the 3-week experiment, P deficiencies are expected to bring plant development to a halt sooner or later.

The choice of plant species depends on availability of inoculum and on whether the main target is biomass production or the removal of a particular nutrient. *Azolla* was easily available and produced more biomass than *Spirodela* – probably also thanks to higher inoculation density. It also removed PO_4-P and K more efficiently, while *Spirodela* achieved higher removal rates for NH_4-N. The ratio of N assimilation by Spirodela being only marginal, it must be assumed that most N was removed through other processes such as denitrification and NH_3 volatilization.

Further Research Needs

If further research were to be carried out, it should address:

- **Nutrient removal**: More reliable results could be achieved with further experiments including more replications. The contribution of plants towards nutrient removal as well as the proportion lost through other processes (NH_3 volatilization, denitrification, precipitation of solid compounds) should be quantified. If more efficient K removal is desired, additional studies on K are necessary.
- **Plant requirements:** Further field studies with urine fertilized aquaculture should focus on limiting factors other than P availability, e.g. micronutrients and pH conditions.
- **Year-round production feasibility and choice of aquatic plants:** The presented field experiments were limited to *Azolla* and *Spirodela* production under the climatic conditions of the Kathmandu Valley from late January to March. The research neither reflects year-round feasibility (e.g. during the rainy season and the coldest weeks of winter) nor does it assess the potential of *Azolla* and *Spirodela* cultivation in other parts of Nepal such as the lower-lying subtropical Terai region.

It is therefore essential to conduct more studies on the performance of these plant species throughout the year. For the Kathmandu Valley, especially the winter season is a critical point because of low temperatures. In the Terai and other parts of the country, climatic conditions are different so that separate research is required. Depending on the region, different species from the (sub)families of *Azollaceae* and *Lemnoideae* or even other native aquatic plants might be better adapted to the respective environment.

- **Potential to upscale production:** From the small production tanks used during the experiment it is difficult to predict the functioning of a larger system. Besides changed management practices, a larger aquaculture also has high land requirements. The availability of otherwise unproductive land in suitable locations should therefore be investigated.

  During the experiment only small amounts of fertilizer (urine, effluent) inputs were needed to reach optimal NH_4-N concentrations so that the question arises whether larger systems would be a real outlet for excess urine or effluent. To what level could the concentrations be increased? Would awareness-raising and promotion of direct urine application or drip irrigation with effluent not be preferable (and possibly more efficient) for nutrient recycling than the “detour” of aquaculture?

- **Assessment of needs and interests:** An essential matter that must be looked into before pursuing the idea of aquaculture is whether there is any need among local farmers for additional fodder, green manure, or compostable biomass. Further concerns are the level of interest in changing or extending agricultural practices and the attitude of the community towards aquaculture. Without any need for biomass, interest in and acceptance of aquaculture, other reuse options for urine are probably better suited.

- **Economic viability**: How would the additional labor input and land requirements compare with possible revenue and/or savings? Is there a market for aquacultural products? Would an integrated system with fish production be a more economically attractive option?

- **Spirulina:** Although considered unsuitable for outdoor cultivation in the Kathmandu Valley, it might be worthwhile, due to its nutritional value, to explore possibilities of building a greenhouse to maintain temperature even in the winter or carry out experiments in the warmer Terai region in the South of Nepal. For spirulina cultivation, a source of spirulina inoculum in Northern India or possibly even within Nepal should be found.

  To assess the feasibility of growing spirulina on urine without long-term deficiencies and with significant reduction of additional chemical fertilizer inputs, experiments should first be conducted in a suitable (tropical) climate to avoid simultaneous examination of too many influencing variables (nutrient availability, climatic conditions, adaptation of spirulina strains, etc.).

  As for *Azolla* and duckweed, a central issue would be the use of harvested spirulina: Would it be safe and acceptable as human food when grown on diluted urine? Would its use as protein-rich animal feed justify the high labor and additional fertilizer inputs? Would it be economically viable?

The field of nutrient recovery from urine through aquatic plants definitely opens a wide range of research topics to be explored in the future.

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Appendix A: Urine / Effluent Sample Analysis Report (ENPHO)

Table 19: Analysis report of urine / effluent samples (ENPHO)

SAMPLE ANALYSIS REPORT

Lab Reg. No. 1060-1061/(066-067) **Code : US**

Client : Martina Karli
Address **:** Pulchowk, Lalitpur
Sampled By **:** Client

Source of Sample : Urine/Effluent
Location/Area **:** Siddhipur, Lalitpur
Received on **:** 12th Jan 2010
Date of Analysis **:** 12th-17th Jan 2010

P H Y S I C O - C H E M I C A L AND M I C R O B I O L O G I C A L A N A L Y S I S

S. No.	Sample ID	Sample ID	Sample ID	Sample ID	Test Method
		Unit	1060	1061	
1	Alkalinity	mg/L	5800	5700	**Acid Titration**
2	Nitrate	mg/L	46.51	24.36	**UV Spectrophotometric (Screening)**
3	Ammonia	mg/L	2360	2212	**Spectrophotometric (Nesslerization)**
4	Calcium	mg/L	20	388	**EDTA Titration**
5	Magnesium	mg/L	<1	61	**EDTA Titration**
6	T. Phosphate	mg/L	318	21.70	**Ammonium molybdate ascorbic acid red^{n}**
7	Orthophosphate	mg/L	292	15.80	**Ammonium molybdate ascorbic acid red^{n}**
8	Potassium	mg/L	523	516	**Atomic Absorption Spectrometer (AAS) (Flame Emission)**
9	Chemical Oxygen Demand (COD)	mg/L	913	632	**Dichromate oxidation and titration with ferrous ammonium sulphate**

Note:

LR:1060 - Urine
LR:1061 - Effluent

Table 20: Conversion into comparable parameters (urine / effluent samples)

C O N V E R S I O N

		Unit	Urine	Effluent	Conversion
	NO_3-N	mg/L	10.507	5.503	**NO_3 · 0.2259**
	NH_4-N	mg/L	1833	1718	**NH_4 · 0.7765**
	PO_4-P	mg/L	95.19	5.15	**PO_4 · 0.3261**
	Proportion of N present as NO_3-N	mg/L	0.57%	0.32%	**NO_3-N / (NH_4-N + NO_3-N)**

Appendix B: Well Water Analysis Report (ENPHO)

Table 21: Analysis report of dug well water, Siddhipur (ENPHO)

WATER ANALYSIS REPORT

Lab Reg. No1054/(066-067) **Code : DW**

Client : Martina Karli	**Source of Sample :** Well
Address **:** Pulchowk, Lalitpur	Location/Area : Shiddipur, Lalitpur
Sampled By **:** Client	Received on **:** 6th Jan 2010
	Date of Analysis **:-** 6-8th Jan 2010

P H Y S I C O - C H E M I C A L A N A L Y S I S

Parameters	Unit	Sample ID	NDWQS	Test Methods
		1054		
Total Hardness as $CaCO_3$	mg/L	140	**500**	**EDTA Titration**
Calcium	mg/L	49.60	**200**	**EDTA Titration**
Magnesium	mg/L	3.88	**-**	**EDTA Titration**
Ammonia	mg/L	0.43	**1.5**	**Spectrophotometric (Nesslerization)**
Nitrate	mg/L	3.51	**50**	**UV Spectrophotometric (Screening)**
Orthophosphate	mg/L	0.11	**-**	**Ammonium molybdate ascorbic acid red^n**
Potassium (K)	mg/L	5.08		**Atomic Absorption Spectrometer (AAS)**
Chemical Oxygen Demand (COD)	mg/L	3	**-**	**Dichromate oxidation and titration with ferrous ammonium sulphate**

NDWQS = National Drinking Water Quality Standard (2062)

Reference : Standard Methods for the Examination of Water and Wastewater (APHA, AWWA & WEF) 19th Edition (1995)
** Not Accredited Parameters*
ND: Not Detected ( ) Maximum Concentration Limit
Remarks: All the tested physico - chemical parameters are within the NDWQS at the time of analysis.

Table 22: Conversion into comparable parameters (well water sample)

C O N V E R S I O N

	Unit	Water	Conversion
NO_3-N	mg/L	0.7929	**NO_3 · 0.2259**
NH_4-N	mg/L	0.3339	**NH_4 · 0.7765**
PO_4-P	mg/L	0.0359	**PO_4 · 0.3261**
Proportion of N present as NO_3-N	mg/L	70.37%	**NO_3-N / (NH_4-N + NO_3-N)**

Appendix C: Results Photospectrometric Urine / Effluent / DAP Analyses

Table 23: Result sheet urine / effluent / DAP analyses

NH_4-N content [mg·L^{-1}] Analysis at UN-Habitat, Nepal; dilution: 1:10000

	23-Feb	03-Mar	03-Mar	03-Mar	10-Mar
Urine - 0.45 filter	2900	700	950	1700	2650
Effluent - 0.45 filter	2500	700	1150	2500	2300
DAP solution	1800	700	550	1600	1700

Annotations:
Using distilled water for car batteries
Repetition with car battery water, dilution 1:5000
Repetition with Aqua Hundred mineral water --> used for calculations
On March 3, urine from a different container was used.

PO_4-P content [mg·L^{-1}] Analysis at UN-Habitat, Nepal

	23-Feb	dilution	03-Mar	dilution	10-Mar	dilution
Urine - 0.45 filter	197.5	1:25	94	1:10	167.5	1:25
Effluent - 0.45 filter	20	1:10	67	1:10	75	1:10
DAP solution	925	1:250	800	1:250	775	1:250

K-content [mg·L^{-1}] Analysis at Eawag, Switzerland; dilution: 1:1000

	23-Feb	03-Mar	10-Mar
Urine - 0.45 filter	573	339	516
Effluent - 0.45 filter	779		
DAP solution	5	5	5

Annotations:
The same effluent was used for all 3 weeks; K values are expected to have remained the same.
DAP solution only contains K from well water.

Appendix D: Results Biomass Production

Table 24: Result sheet biomass production

Biomass [g]	initial fresh matter 24-Feb	initial dry matter (K_o)	final fresh matter 17-Mar	fresh matter increase	Conver-sion factor	final dry matter (K_t), 17-Mar	dry matter increase	increase in % (comp. to initial dry matter)
Azolla / Urine								
4	60	2.5860	297	237	0.0444	13.1868	10.5228	407%
12	60	2.5860	315	255	0.0444	13.9860	11.3220	438%
15	60	2.5860	290	230	0.0444	12.8760	10.2120	395%
average	60		301	241		13.3496	10.6856	413%
st.dev			13	13				0.22
st.error			7	7				0.13
Azolla / Effluent								
7	60	2.5860	296	236	0.0495	14.6520	11.6820	452%
8	60	2.5860	295	235	0.0495	14.6025	11.6325	450%
18	60	2.5860	297	237	0.0495	14.7015	11.7315	454%
average	60		296	236		14.6520	11.6820	452%
st.dev			1	1				0.02
st.error			1	1				0.01
Azolla / E + DAP								
1	60	2.5860	245	185	0.0431	10.5595	7.9735	308%
10	60	2.5860	264	204	0.0431	11.3784	8.7924	340%
16	60	2.5860	259	199	0.0431	11.1629	8.5769	332%
average	60		256	196		11.0336	8.4476	327%
st.dev			10	10				0.16
st.error			6	6				0.09
Spirodela / Urine								
2	22	1.2782	92.5	70.5	0.0553	5.1153	3.8987	305%
3	22	1.2782	82.5	60.5	0.0553	4.5623	3.3457	262%
17	22	1.2782	83	61	0.0553	4.5899	3.3733	264%
average	22		86	64		4.7558	3.5392	277%
st.dev			6	6				0.24
st.error			3	3				0.14
Spirodela / Effluent								
5	22	1.2782	77	55	0.0623	4.7971	3.4265	268%
11	22	1.2782	67	45	0.0623	4.1741	2.8035	219%
13	22	1.2782	67	45	0.0623	4.1741	2.8035	219%
average	22		70	48		4.3818	3.0112	236%
st.dev			6	6				0.28
st.error			3	3				0.16
Spirodela / E + DAP								
6	22	1.2782	66	44	0.0581	3.8346	2.5564	200%
9	22	1.2782	57	35	0.0581	3.3117	2.0335	159%
14	22	1.2782	77.5	55.5	0.0581	4.5028	3.2246	252%
average	22		67	45		3.8830	2.6048	204%
st.dev			10	10				0.47
st.error			6	6				0.27

Table 25: N content of *Spirodela*

	average dry matter increase per tank	total N content (KU analysis)	assimilated N [mg]	total N used per tank (average) [mg]	taken up by plants
Spirodela / Urine	3.5392	0.62%	21.94	1316	1.67%
Spirodela / Effluent	3.0112	1.43%	43.06	1536	2.80%
Spirodela / E + DAP	2.6048	0.56%	14.59	1389	1.05%

Appendix E: Result Sheets Nutrient Removal Analyses

Table 26: Result sheets ammonium measurements

Ammonium (NH_4-N)

Week 8/9 treatment, tank no.	24-Feb	probably falsified 03-Mar	corrective estimate 03-Mar	before fertilization		estimate used NH_4-N week 8/9	fertilization 04-Mar			fertilization 05-Mar		estimate N cont 5-Mar	added NH_4-N since beginning
Azolla / Urine	abs conc (calc)	mg/l	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc.)	mg	added urine		added water	added urine		mg	mg
4	711	11	24	31.0	735	-24	0.100		3.900	0.066		1018	994
12	711	3	6	30.9	199	511	0.100		4.000	0.066		483	994
15	711	5	11	31.1	334	377	0.100		3.850	0.066		617	994
average			8.60			444	0.100		3.917	0.066		550	994
Azolla / Effluent							added effluent			added effluent			
7	712	4.5	10	31.0	301	411	0.100		3.900	0.065		715	1125
8	712	6	13	30.9	399	313	0.100		4.000	0.065		812	1125
18	712	3	6	30.8	199	513	0.100		4.100	0.065		613	1125
average			9.68			412	0.100		4.000	0.065		713	1125
Azolla / E + DAP							added effluent	added DAP		added effluent	added DAP		
1	712	17	17	30.9	525	186	0.100	0.000	4.000	0.050	0.000	902	1088
10	712	6	12	31.3	376	336	0.100	0.000	3.600	0.050	0.000	752	1088
16	712	2	4	31.0	133	578	0.100	0.000	3.900	0.050	0.000	510	1088
average			11.10			367	0.100	0.000	3.833	0.050	0.000	721	1088
Spirodela / Urine										added urine			
2	711	7.5	16	30.9	494	216	0.100		4.000	0.066		778	994
3	711	12	25	30.9	773	-62	0.100		4.000	0.066		1056	994
17	711	3	6	30.9	199	511	0.100		4.000	0.066		483	994
average			11.23			364	0.100		4.000	0.066		630	994
Spirodela / Effluent							added effluent			added effluent			
5	712	3	7	31.0	202	510	0.100		3.900	0.065		615	1125
11	712	4	9	30.8	265	447	0.100		4.100	0.065		679	1125
13	712	4	9	31.0	267	445	0.100		3.900	0.065		680	1125
average			7.90			467	0.100		3.967	0.065		658	1125
Spirodela / E + DAP							added effluent	added DAP		added effluent	added DAP		
6	712	4	9	31.1	267	445	0.100	0.000	3.850	0.050	0.000	643	1088
9	712	4	9	31.0	267	445	0.100	0.000	3.900	0.050	0.000	643	1088
14	712	5	10.75	31.0	333	378	0.100	0.000	3.900	0.050	0.000	710	1088
average			9.32			423	0.100	0.000	3.883	0.050	0.000	665	1088

Mar 03

NH_4-N content urine: 1700 $mg \cdot L^{-1}$ NH_4-N content effluent: 2500 $mg \cdot L^{-1}$ NH_4-N content DAP: 1600 $mg \cdot L^{-1}$ NH_4-N content water: 0.3339 $mg \cdot L^{-1}$

highlighted values signify NH_4-N increase instead of removal

Ammonium (NH_4-N) (continued)

WEEK 10 treatment, tank no.	estimate N cont 5-Mar	added NH_4-N since beginning	10-Mar	before fertilization		used NH_4-N week 9/10	fertilization 11-Mar			fertilization 12-Mar			N cont 12-Mar	used NH_4-N since beginning
Azolla / Urine	mg	mg	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc.)	mg	added urine		added water	added urine			mg	mg
4	1018	994	8	30.7	246	773	0.103		4.200	0.102			790	748
12	483	994	3	30.3	91	392	0.103		4.600	0.102			636	903
15	617	994	5	30.8	154	463	0.103		4.100	0.102			699	840
average	706	994	5			543			4.300				708	831
Azolla / Effluent							added effluent			added effluent				
7	715	1125	14	30.2	423	292	0.100		4.700	0.070			815	703
8	812	1125	10	30.6	306	506	0.100		4.300	0.070			698	819
18	613	1125	6	30.2	181	431	0.100		4.700	0.070			574	944
average	713	1125	10			410			4.567				696	822
Azolla / E + DAP							added effluent	added DAP		added effluent	added DAP			
1	902	1088	7	30.9	216	686	0.100	0.025	4.000	0.078	0.018		700	872
10	752	1088	12	30.9	371	381	0.100	0.025	4.000	0.078	0.018		854	717
16	510	1088	6	30.1	180	329	0.100	0.025	4.800	0.078	0.018		665	907
average	721	1088	8			381			4.267				740	832
Spirodela / Urine							added urine			added urine				
2	778	994	14	30.1	421	357	0.103		4.800	0.102			966	573
3	1056	994	5	30.6	153	903	0.103		4.300	0.102			698	841
17	483	994	4	29.9	120	363	0.103		5.000	0.102			665	874
average	772	994	8			541			4.700				776	763
Spirodela / Effluent							added effluent			added effluent				
5	615	1125	6	30.3	189	426	0.120		4.600	0.103			704	936
11	679	1125	5	30.1	150	528	0.120		4.800	0.103			665	975
13	680	1125	6	30.2	181	499	0.120		4.700	0.103			696	944
average	658	1125	6			499			4.700				688	952
Spirodela / E + DAP							added effluent	added DAP		added effluent	added DAP			
6	643	1088	7	30.3	212.4	431	0.100	0.054	4.500	0.033	0.050		697	875
9	643	1088	6	30.3	182	461	0.100	0.059	4.500	0.033	0.045		666	906
14	710	1088	9	30.3	273	436	0.100	0.054	4.500	0.033	0.050		757	815
average	665	1088	7			443			4.500				707	865

Mar 10

NH_4-N content urine: 2650 $mg \cdot L^{-1}$ NH_4-N content effluent: 2300 $mg \cdot L^{-1}$ NH_4-N content DAP: 1700 $mg \cdot L^{-1}$ NH_4-N content water: 0.3339 $mg \cdot L^{-1}$

Ammonium (NH_4-N) (continued)

WEEK 11 treatment, tank no.	N cont 12-Mar	added NH_4-N since beginning	17-Mar			used NH_4-N week 9/10	used NH_4-N since beginning	% of NH_4-N used
Azolla / Urine	mg	mg	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc.)	mg	mg	
4	789	1539	10	31.5	315	474	1224	79.5%
12	634	1539	8	31.2	234	400	1305	84.8%
15	697	1539	9	31.3	266	431	1273	82.7%
average	707	1539	8.67	31.33	271.68	435.05	1267	82.3%
Azolla / Effluent				Volume				
7	814	1518	7	31.5	221	593	1297	85.5%
8	697	1518	8	31.1	249	448	1269	83.6%
18	572	1518	10	31.2	312	260	1206	79.4%
average	694	1518	8.33	31.27	260.43	433.90	1258	82.8%
Azolla / E + DAP								
1	699	1572	11	31.3	344	354	1227	78.1%
10	853	1572	11	31	341	512	1231	78.3%
16	663	1572	11	31.4	345	318	1227	78.0%
average	738	1572	11	31	344	395	1228	78.1%
Spirodela / Urine								
2	965	1539	8	31.1	233	731	1306	84.8%
3	696	1539	11	31	326	371	1213	78.8%
17	663	1539	4	31	109	554	1430	92.9%
average	775	1539	7	31	222	552	1316	85.5%
Spirodela / Effluent								
5	702	1640	3	31.5	95	608	1545	94.2%
11	663	1640	4	31	109	555	1531	93.4%
13	694	1640	4	31.2	109	585	1531	93.3%
average	686	1640	3	31	104	582	1536	93.7%
Spirodela / E + DAP								
6	695	1572	9	31.3	282	413	1290	82.1%
9	665	1572	4	31.3	110	555	1463	93.0%
14	756	1572	5	31.3	157	599	1416	90.0%
average	705	1572	6	31	183	523	1389	88.4%

Table 27: Result sheets phosphate measurements

Phosphate (PO_4-P)

WEEK 8/9												
treatment, tank no.	P cont 24-Feb	03-Mar	before fertilization		used PO_4-P week 8/9	fertilization 04-Mar			fertilization 05-Mar		P cont 05-Mar	added PO_4-P since beginning
Azolla / Urine	abs cont (calc)	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc)		added urine		added water	added urine		mg	mg
4	48.8	0.6	31.0	17.1	31.8	0.100		3.900	0.066		32.8	64.6
12	48.8	0.6	30.9	18.5	30.3	0.100		4.000	0.066		34.3	64.6
15	48.8	0.6	31.1	17.1	31.8	0.100		3.850	0.066		32.8	64.6
average		0.6		17.6	31.3	0.100		3.917	0.066		33.3	64.6
Azolla / Effluent						added effluent			added effluent			
7	6.8	0.5	31.0	15.5	-8.7	0.100		3.900	0.065		26.7	18.0
8	6.8	0.4	30.9	12.4	-5.5	0.100		4.000	0.065		23.6	18.0
18	6.8	0.3	30.8	9.2	-2.4	0.100		4.100	0.065		20.4	18.0
average		0.4		12.4	-5.5	0.100		4.000	0.065		23.6	18.0
Azolla / E + DAP						added effluent	added DAP		added effluent	added DAP		
1	143.4	4.2	30.9	129.8	13.7	0.100	0.000	4.000	0.050	0.000	140.0	153.6
10	143.4	4.2	31.3	132.2	11.2	0.100	0.000	3.600	0.050	0.000	142.4	153.6
16	143.4	4.2	31.0	130.2	13.2	0.100	0.000	3.900	0.050	0.000	140.4	153.6
average		4.2		130.7	12.7	0.100	0.000	3.833	0.050	0.000	140.9	153.6
Spirodela / Urine						added urine			added urine			
2	48.8	1.1	30.9	34.0	14.9	0.100		4.000	0.066		49.7	64.6
3	48.8	1.1	30.9	34.8	14.1	0.100		4.000	0.066		50.5	64.6
17	48.8	1.1	30.9	34.0	14.9	0.100		4.000	0.066		49.7	64.6
average		1.1		34.2	14.6	0.100		4.000	0.066		50.0	64.6
Spirodela / Effluent						added effluent			added effluent			
5	6.8	0.9	31.0	27.9	-21.1	0.100		3.900	0.065		39.1	18.0
11	6.8	0.9	30.8	27.7	-20.9	0.100		4.100	0.065		38.9	18.0
13	6.8	0.9	31.0	27.9	-21.1	0.100		3.900	0.065		39.1	18.0
average		0.9		27.8	-21.0	0.100		3.967	0.065		39.0	18.0
Spirodela / E + DAP						added effluent	added DAP		added effluent	added DAP		
6	143.4	4.3	31.1	132.0	11.5	0.100	0.000	3.850	0.050	0.000	142.2	153.6
9	143.4	4.3	31.0	134.1	9.4	0.100	0.000	3.900	0.050	0.000	144.3	153.6
14	143.4	3.9	31.0	120.9	22.5	0.100	0.000	3.900	0.050	0.000	131.1	153.6
average		4.2		129.0	14.5	0.100	0.000	3.883	0.050	0.000	139.2	153.6

Mar 03

PO_4-P content urine: 94 $mg \cdot L^{-1}$ PO_4-P content effluent: 67 $mg \cdot L^{-1}$ PO_4-P content DAP: 800 $mg \cdot L^{-1}$ PO_4-P content water: 0.0359 $mg \cdot L^{-1}$

highlighted values signify PO_4-P increase instead of removal

Phosphate (PO_4-P) (continued)

WEEK 10					used PO_4-P					P cont 12-	used PO_4-P since
treatment, tank no.	P cont 05-Mar	10-Mar	before fertilization		week 9/10	fertilization 11-Mar			fertilization 12-Mar	Mar	beginning
Azolla / Urine	mg	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc)	mg	added urine	added water		added urine	mg	mg
4	32.8	0.5	30.7	15.3	17.4	0.103	4.200		0.102	49.8	49.2
12	34.3	0.4	30.3	12.1	22.2	0.103	4.600		0.102	46.6	52.5
15	32.8	0.7	30.8	21.6	11.3	0.103	4.100		0.102	56.0	43.0
average	33.3	0.5		16.3	17.0		4.300			50.8	48.2
Azolla / Effluent						added effluent			added effluent		
7	26.7	0.5	30.2	15.1	11.6	0.100	4.700		0.070	28.0	2.9
8	23.6	0.4	30.6	12.2	11.3	0.100	4.300		0.070	25.1	5.8
18	20.4	0.5	30.2	14.3	6.1	0.100	4.700		0.070	27.3	3.7
average	23.6	0.5		13.9	9.7		4.567			26.8	4.2
Azolla / E + DAP						added effluent	added DAP		added effluent	added DAP	
1	140.0	3.0	30.9	92.6	47.3	0.100	0.025	4.000	0.078	0.018	139.4	61.0
10	142.4	3.1	30.9	95.7	46.7	0.100	0.025	4.000	0.078	0.018	142.5	57.9
16	140.4	2.9	30.1	87.2	53.2	0.100	0.025	4.800	0.078	0.018	134.1	66.4
average	140.9	3.0		91.9	49.1			4.267			138.7	61.8
Spirodela / Urine						added urine			added urine		
2	49.7	1.0	30.1	30.1	19.6	0.103	4.800		0.102	64.6	34.5
3	50.5	0.6	30.6	18.4	32.2	0.103	4.300		0.102	52.9	46.2
17	49.7	0.8	29.9	23.9	25.8	0.103	5.000		0.102	58.4	40.7
average	50.0	0.8		24.1	25.9		4.700			58.6	40.5
Spirodela / Effluent						added effluent			added effluent		
5	39.1	0.9	30.3	27.3	11.8	0.120	4.600		0.103	44.1	-9.2
11	38.9	0.7	30.1	21.1	17.9	0.120	4.800		0.103	38.0	-3.0
13	39.1	0.5	30.2	15.1	24.0	0.120	4.700		0.103	32.0	3.0
average	39.0	0.7		21.1	17.9		4.700			38.0	3.0
Spirodela / E + DAP						added effluent	added DAP		added effluent	added DAP	
6	142.2	1.2	30.3	36.4	105.7	0.100	0.054	4.500	0.033	0.050	127.2	117.2
9	144.3	1.8	30.3	54.6	89.7	0.100	0.059	4.500	0.033	0.045	145.4	99.0
14	131.1	1.6	30.3	48.6	82.5	0.100	0.054	4.500	0.033	0.050	139.3	105.1
average	139.2	1.5		46.5	92.6			4.500			137.3	107.1

Mar 10

PO_4-P content urine: 167.5 $mg \cdot L^{-1}$ PO_4-P content effluent: 75 $mg \cdot L^{-1}$ PO_4-P content DAP: 775 $mg \cdot L^{-1}$ PO_4-P content water: 0.0359 $mg \cdot L^{-1}$

highlighted values signify PO_4-P increase instead of removal

Phosphate (PO_4-P) (continued)

WEEK 11 treatment, tank no.	P cont 12-Mar	added PO_4-P since beginning	17-Mar			used PO_4-P week 10/11	total used PO_4-P since beginning	% of PO_4-P used
Azolla / Urine	mg	mg	$mg \cdot L^{-1}$	vol (meas.)	abs cont		mg	
4	49.8	99.1	0.9	31.5	28.4	21.5	70.7	71.4%
12	46.6	99.1	0.8	31.2	25.0	21.7	74.1	74.8%
15	56.0	99.1	0.9	31.3	28.2	27.9	70.9	71.6%
average	50.8	99.1	0.9	31.3	27.2	23.7	71.9	72.6%
Azolla / Effluent								
7	28.0	31.0	0.7	31.5	22.1	6.0	8.9	28.8%
8	25.1	30.9	0.7	31.1	21.8	3.4	9.2	29.7%
18	27.3	31.0	0.6	31.2	18.7	8.5	12.2	39.5%
average	26.8	31.0	0.7	31.3	20.8	6.0	10.1	32.7%
Azolla / E + DAP								
1	139.4	200.4	4.6	31.3	144.0	-4.5	56.5	28.2%
10	142.5	200.4	4.5	31.0	139.5	3.0	60.9	30.4%
16	134.1	200.5	4.5	31.4	141.3	-7.2	59.2	29.5%
average	138.7	200.5	4.5	31.2	141.6	3.0	60.9	30.4%
Spirodela / Urine								
2	64.6	99.1	1.2	31.1	37.3	27.3	61.8	62.3%
3	52.9	99.1	1.6	31.0	49.6	3.3	49.5	49.9%
17	58.4	99.1	1.5	31.0	46.5	11.9	52.6	53.1%
average	58.6	99.1	1.4	31.0	44.5	14.2	54.6	55.1%
Spirodela / Effluent								
5	44.1	34.9	0.9	31.5	28.4	15.8	6.6	18.8%
11	38.0	34.9	1.1	31.0	34.1	3.9	0.8	2.4%
13	32.0	34.9	1.0	31.2	31.2	0.8	3.7	10.7%
average	38.0	34.9	1.0	31.2	31.2	6.8	5.2	14.8%
Spirodela / E + DAP								
6	127.2	244.4	2.9	31.3	89.2	37.9	155.2	63.5%
9	145.4	244.4	4.4	31.3	137.7	7.6	106.6	43.6%
14	139.3	244.4	3.6	31.3	112.7	26.6	131.7	53.9%
average	137.3	244.4	3.6	31.3	113.2	24.1	131.2	53.7%

highlighted values signify PO_4-P increase instead of removal

Table 28: Result sheets K measurements

Potassium (K)

treatment, tank no.	K cont 24-Feb	03-Mar	before fertilization		used K week 8/9	fertilization 04-Mar			fertilization 05-Mar		K cont 05-Mar	added K since beginning
Azolla / Urine	abs cont (calc)	mg·L^{-1}	vol (meas.)	abs cont (calc)	mg	added urine		added water	added urine		mg	mg
15	314.7	8.7	31.1	270	44.5	0.100		3.850	0.066		346.0	390.5
Azolla / Effluent						added effluent			added effluent			
18	394.5	10.0	30.8	308	86.5	0.100		4.100	0.065		457.4	523.0
Azolla / E + DAP						added effluent	added DAP		added effluent	added DAP		
10	310.2	7.1	31.3	222	87.9	0.100	0.000	3.600	0.050	0.000	357.4	427.0
Spirodela / Urine						added urine			added urine			
3	314.7	11.2	30.9	346	-31.4	0.100		4.000	0.066		422.7	370.9
Spirodela / Effluent						added effluent			added effluent			
13	394.5	12.2	31.0	378	16.3	0.100		3.900	0.065		526.5	523.0
Spirodela / E + DAP						added effluent	added DAP		added effluent	added DAP		
9	310.2	9.7	31.0	301	9.5	0.100	0.000	3.900	0.050	0.000	437.4	427.0

Mar 03
K content urine: 339 mg·L^{-1} K content effluent: 779 mg·L^{-1} K content DAP: 5.08 mg·L^{-1} K content water: 5.08 mg·L^{-1}

Potassium (K) (continued)

Week 10 treatment, tank no.	K cont 05-Mar	10-Mar	before fertilization		used K week 9/10	fertilization 11-Mar			fertilization 12-Mar		K cont 12-Mar	used K since beginning
Azolla / Urine	mg	mg·L^{-1}	vol (meas.)	abs cont (calc)	mg	added urine		added water	added urine		mg	mg
15	346.0	9.2	30.8	283	62.6	0.103		4.100	0.102		409.9	107.2
Azolla / Effluent						added effluent			added effluent			
18	457.4	12.3	30.2	371	85.9	0.100		4.700	0.070		527.8	151.6
Azolla / E + DAP						added effluent	added DAP		added effluent	added DAP		
10	357.4	8.6	30.9	266	91.8	0.100	0.025	4.000	0.078	0.018	424.6	161.5
Spirodela / Urine						added urine			added urine			
3	422.7	13.3	30.6	407	15.7	0.103		4.300	0.102		534.6	-36.0
Spirodela / Effluent						added effluent			added effluent			
13	526.5	17.2	30.2	519	7.5	0.120		4.700	0.103		716.7	3.9
Spirodela / E + DAP						added effluent	added DAP		added effluent	added DAP		
9	437.4	14.7	30.3	446	-8.7	0.100	0.059	4.500	0.033	0.045	572.8	-19.0

Mar 10
K content urine: 516 mg·L^{-1} K content effluent: 779 mg·L^{-1} K content DAP: 5.08 mg·L^{-1} K content water: 5.08 mg·L^{-1}
highlighted values signify K increase instead of removal

Potassium (K) **(continued)**

Week 11 treatment, tank no.	K cont 12-Mar	added K since beginning	17-Mar			used K week 10/11	total used K since beginning	% of K used
Azolla / Urine	mg	mg	$mg \cdot L^{-1}$	vol (meas.)	abs cont (calc)	mg	mg	
15	409.9	517.1	15.3	31.3	479	-68.9	38.2	7.4%
Azolla / Effluent				Volume				
18	527.8	679.3	16.9	31.2	527	0.5	152.1	22.4%
Azolla / E + DAP								
10	424.6	586.0	14.7	31	456	-31.1	130.3	22.2%
Spirodela / Urine								
3	534.6	498.6	19.7	31	611	-76.1	-112.1	-22.5%
Spirodela / Effluent								
13	716.7	720.6	21.9	31.2	683	33.4	37.3	5.2%
Spirodela / E + DAP								
9	572.8	553.5	17.1	31.3	535	37.5	18.2	3.3%

highlighted values signify PO_4-P increase instead of removal

Appendix F: Standard Addition for NH_4-N Analysis (Salicylate Method)

Date 18-Mar-10
Machine HACH DR 2000
Matrix diluted urine
Standard NH_4-standard (1000mg·L^{-1} NH_4^+)

Table 29: Linear calibration curve (Etter, 2009)

Standard-Nr.	reading y_i [-]	target concentration x_i [mg·L^{-1} N]	measurement [mg·L^{-1} N]
1	0.54	0.54	0.55
2	0.45	0.48	0.45
3	0.43	0.41	0.43
4	0.35	0.34	0.35
5	0.27	0.27	0.26
6	0.22	0.20	0.21
7	0.14	0.14	0.13
8	0.08	0.07	0.07

Method standard deviation: s_{x0} = **0.015** **[mg·L^{-1} N]**

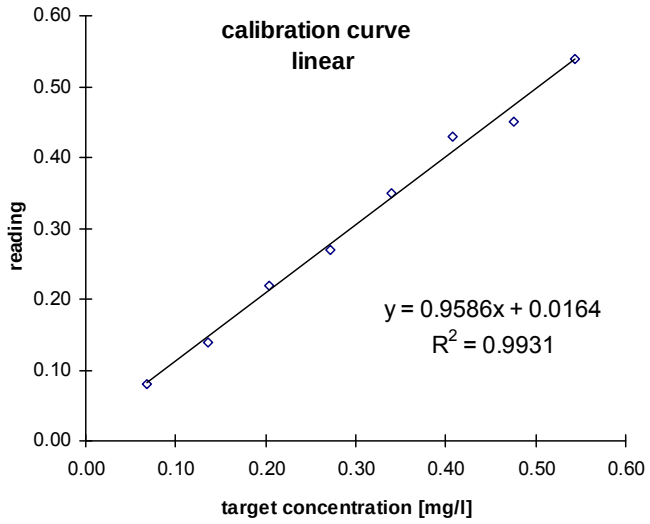

Fig. 43: Calibration curve, NH_4^+ salicylate method (Etter, 2009)

Table 30: Sample preparation for standard addition

NH_4-standard (1000mg·L^{-1} NH_4^+)	776.50	mg·L^{-1} N
dilution	1553.00	
NH_4-concentration of stock solution	0.50	mg·L^{-1} N
NH_4-concentration of sample	0.30	mg·L^{-1} N

Standard Nr.	sample (diluted urine) [ml]	stock solution [ml]	reference value [mg·L^{-1} N]
1	24	0	0.3000
2	21	3	0.3250
3	18	6	0.3500
4	15	9	0.3750
5	12	12	0.4000
6	9	15	0.4250
7	6	18	0.4500
8	3	21	0.4750

Table 31: Standard addition

Addition-Nr.	reading Y [-]	actual value X_{actual} [mg·L^{-1} N]	Δ_{target} [mg·L^{-1} N]	Δ_{actual} [mg·L^{-1} N]
1	0.30	0.30	0.000	0.00
2	0.36	0.36	0.025	0.06
3	0.36	0.36	0.050	0.06
4	0.40	0.40	0.075	0.10
5	0.42	0.42	0.100	0.13
6	0.47	0.47	0.125	0.18
7	0.49	0.49	0.150	0.20
8	0.50	0.50	0.175	0.21

Standard deviation of retrieval function: s_{yf} = **0.013** **[mg·L^{-1} N]**

Coefficient of retrieval function: $\Delta_{actual} = \beta * \Delta_{soll} + \alpha$

		95%-confidence interval lower	upper
β =	**1.12**	0.86	1.37
α =	**0.02**	-0.01	0.05

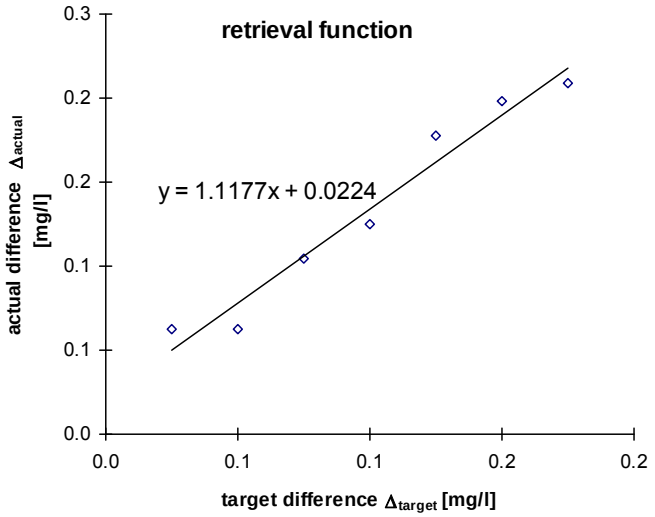

Fig. 44: Retrieval function from NH_4^+ standard addition

Appendix G: Result Sheets Temperature and pH Measurements

Table 32: Result sheet air/water temperature measurements (experiments)

	preliminary experiment								main experiment								
date	3-Feb	05-Feb	10-Feb	12-Feb	15-Feb	17-Feb	19-Feb	22-Feb	26-Feb	01-Mar	03-Mar	05-Mar	08-Mar	10-Mar	12-Mar	15-Mar	17-Mar
tank no. / air temp	8.9	11.6	9.2	8.6	11.6	13.8	15.0	14.8	13.6	18.6	14.5	17.6	16.1	19.3	20.0	18.5	20.5
1	8.1	9.3	8.0	7.8	9.3	9.3	9.6	11.1	10.3	13.6	12.9	13.1	13.4	13.7	14.4	12.9	14.5
2	7.6	9.2	7.7	7.6	8.7	9.6	9.7	11.1	10.5	13.2	13.3	13.2	13.3	13.3	14.4	12.6	14.7
3	7.6	7.8	7.9	7.5	7.9	9.6	9.6	11.2	10.4	13.4	13.2	13.0	13.6	13.4	14.4	12.7	14.7
4	7.0	7.7	7.7	7.5	7.9	9.6	9.4	11.2	10.3	13.4	13.2	13.0	13.4	13.5	14.6	13.0	14.8
5	7.1	8.0	7.8	7.7	8.7	9.9	9.5	11.2	10.5	13.8	13.1	13.3	13.9	13.6	14.8	13.0	14.8
6	7.6	8.9	7.8	7.6	9.1	10.0	10.1	11.4	10.6	13.5	13.4	13.1	13.2	13.8	14.7	12.9	14.6
7	7.9	8.3	7.8	7.6	8.9	10.0	9.8	11.5	10.9	13.5	12.9	13.2	13.6	13.7	15.2	13.1	14.9
8	7.6	8.1	7.8	7.5	8.6	10.4	10.0	11.3	11.0	13.4	13.3	13.2	13.7	13.6	15.0	13.0	14.7
9	7.5	8.2	7.9	7.7	8.9	10.3	10.5	11.6	10.6	14.0	13.2	12.9	13.6	13.6	15.1	13.0	14.7
10	7.4	8.0	8.0	7.9	8.5	10.3	10.4	11.4	11.1	13.7	13.2	13.3	13.6	13.8	14.8	13.1	14.7
11	7.9	8.5	7.9	7.8	8.7	10.7	10.3	11.5	10.6	13.9	13.4	13.3	13.6	13.8	15.2	13.1	15.7
12	7.8	8.8	8.1	7.6	8.8	10.2	10.2	11.6	10.8	13.6	13.2	12.9	13.5	13.5	15.3	13.4	15.4
13	8.1	8.3	8.0	7.7	9.0	10.7	10.2	11.4	10.8	13.8	13.5	13.0	13.7	13.7	15.0	13.3	15.6
14	7.7	8.3	8.0	8.1	8.3	10.4	10.4	11.6	10.7	13.8	13.8	13.4	13.6	13.7	14.9	13.4	15.5
15	8.0	9.0	7.7	7.9	9.3	11.3	11.0	11.8	11.3	13.5	14.0	13.7	13.9	14.3	15.1	13.6	15.9
16	8.8	9.3	8.0	8.0	9.5	11.8	11.0	12.0	11.1	14.2	13.9	13.5	14.3	14.6	15.2	13.8	15.8
17	8.6	9.3	8.0	7.9	9.5	11.5	11.3	11.7	11.0	13.8	14.1	13.4	13.9	14.4	15.2	13.7	16.2
18	8.6	9.0	7.9	8.0	8.7	11.2	10.8	11.7	10.9	13.8	14.0	13.0	14.2	14.3	15.1	13.9	16.0
19				7.8	9.3	11.3	11.5	11.6	12.8	14.1	14.8	13.9	14.7	14.9	15.8	14.5	17.1
average (no.1-18)	7.8	8.6	7.9	7.7	8.8	10.4	10.2	11.5	10.7	13.7	13.4	13.2	13.7	13.8	14.9	13.2	15.2
stdev	0.49	0.55	0.12	0.19	0.47	0.71	0.56	0.25	0.29	0.25	0.38	0.22	0.29	0.36	0.30	0.37	0.57
			min.			max.											

Table 33: Result sheet air/water temperature measurements (preculture)

[°C]	**preculture**	
date	26-Jan	28-Jan
tank no. / air temp	10.1	10.7
1 (Azolla)	8.4	9.0
2 (Azolla)	8.2	8.7
3 (Spirodela)	8.0	8.0
4 (Spirodela)	8.1	7.9
average (no.1-4)	8.2	8.4
stdev.	0.17	0.54

Table 34: Result sheet 24-hour temperature measurement March 4/5, 2010

[°C]	4-Mar																			05-Mar				
time	10:30	11:30	12:30	13:30	14:30	15:30	16:30	17:30	18:30	19:30	20:30	21:30	22:30	23:30	00:30	01:30	02:30	03:30	04:30	05:30	06:30	07:30	08:30	09:30	10:30
tank no. / air temp	17.8	21.5	22.6	25.6	24.4	24.1	22.5	19.7	15.4	14.0	13.2	11.4	10.5	10.6	8.9	8.2	8.2	7.0	6.7	6.3	6.1	10.0	14.9	17.6	22.8
1	14.8	18.9	21.5	23.7	25.6	25.8	25.8	24.5	22.5	20.8	19.9	18.8	17.4	16.2	15.4	14.6	14.0	13.2	12.2	11.8	11.2	10.9	11.6	13.1	16.3
2	15.7	19.3	22.5	24.9	26.6	27.0	26.8	25.4	23.5													10.6	11.3	13.2	16.6
3	15.2	19.4	22.4	24.6	26.4	26.8	26.8	25.3	23.6													10.7	11.4	13.0	16.4
4	14.7	18.6	21.3	23.6	25.2	26.3	25.8	24.3	22.8													10.8	11.4	13.0	16.1
5	15.3	19.3	22.5	24.7	26.6	27.4	26.7	25.0	23.2													10.6	11.2	13.3	16.3
6	15.4	19.6	22.5	24.7	26.5	26.9	26.6	24.9	23.0	21.4	20.1	18.6	17.4	16.5	15.6	14.6	13.8	13.0	12.2	11.6	11.0	10.7	11.5	13.1	16.7
7	14.9	19.0	21.9	23.6	26.4	26.5	26.6	25.3	23.6													11.0	11.5	13.2	16.0
8	14.8	18.8	21.6	23.1	25.3	26.4	26.5	24.9	23.2													10.9	11.3	13.2	16.0
9	15.5	19.3	22.3	24.5	26.2	26.9	26.5	24.9	22.9	21.4	20.1	18.6	17.3	16.4	15.4	14.5	13.7	12.9	12.1	11.5	10.9	10.6	11.2	12.9	16.2
10	15.3	18.8	21.8	23.3	25.7	27.2	26.7	25.3	23.5													10.8	11.7	13.3	15.7
11	16.3	19.6	22.8	25.0	26.4	27.1	26.8	25.2	23.3													10.7	11.4	13.3	16.7
12	16.3	19.0	21.9	23.1	25.9	26.5	26.5	25.1	23.4	21.8	20.4	18.9	17.6	16.7	15.8	14.8	14.1	13.3	12.5	11.9	11.3	10.9	11.4	12.9	16.7
13	15.6	19.3	22.4	24.5	26.4	26.9	26.6	25.0	23.1													10.6	11.3	13.0	16.6
14	15.5	19.0	22.3	24.6	26.5	27.1	26.8	25.1	23.2													10.6	11.2	13.4	16.1
15	15.3	18.7	22.0	23.6	25.9	27.2	26.9	25.2	23.4													10.9	11.8	13.7	16.4
16	16.2	19.4	22.4	24.6	26.3	27.2	26.9	25.3	23.5													10.9	11.7	13.5	17.0
17	16.4	19.6	22.7	24.9	26.5	27.0	26.5	24.9	23.0													10.6	11.5	13.4	17.0
18	16.1	18.7	21.8	23.6	25.9	26.8	26.4	24.8	23.1													10.8	11.8	13.0	16.3
19	16.5	19.6	23.4	25.1	26.7	27.2	26.6	24.7	22.7	20.9	19.6	18.1	16.9	16.0	15.1	14.2	13.3	12.5	11.7	11.2	10.6	10.3	11.5	13.9	16.9
average (no.1-18)	15.5	19.1	22.1	24.1	26.1	26.8	26.6	25.0	23.2	21.4	20.1	18.7	17.4	16.5	15.6	14.6	13.9	13.1	12.3	11.7	11.1	10.8	11.5	13.2	16.4

Table 35: pH measurements

pH	preliminary experiment								main experiment			
tank no.	3-Feb	05-Feb	10-Feb	12-Feb	15-Feb	17-Feb	19-Feb	22-Feb	26-Feb	01-Mar	03-Mar	05-Mar - 17-Mar
1	8.09	8.11	8.70	9.17	8.60	9.00	8.56	8.85	8.05	7.88	7.99	unreadable display
2	8.25	8.20	8.75	9.02	8.78	9.03	8.69	9.18	8.16	8.06	8.44	
3	8.21	8.22	8.70	9.02	8.85	8.99	8.70	9.13	8.16	8.05	8.40	
4	8.23	8.23	8.78	9.03	8.89	9.02	8.66	8.88	8.17	7.90	7.98	
5	8.32	8.30	8.64	8.91	8.84	8.98	8.70	8.99	8.16	8.00	8.41	
6	8.40	8.29	8.57	8.80	8.81	9.18	8.79	9.03	8.01	8.10	8.55	
7	8.50	8.36	8.40	8.71	8.74	calibr error	8.85	9.00	8.16	7.93	8.12	
8	8.47	8.38	8.40	8.56	8.78	calibr error	8.79	8.98	8.15	7.88	8.00	
9	8.30	8.29	8.56	8.56	8.83	calibr error	8.92	9.04	8.01	8.00	8.26	
10	8.10	8.17	8.63	8.82	8.86	calibr error	8.77	8.78	8.01	7.92	7.94	
11	8.40	8.34	8.50	8.77	8.77	calibr error	8.78	9.13	8.09	8.06	8.53	
12	8.40	8.31	8.51	8.88	8.88	calibr error	8.94	9.10	8.13	7.90	7.88	
13	8.30	8.27	8.55	8.81	8.78	calibr error	8.68	9.08	8.11	7.98	8.47	
14	8.08	8.17	8.56	8.80	8.80	calibr error	8.77	9.10	7.98	8.11	8.88	
15	8.37	8.30	8.52	8.88	8.93	calibr error	8.91	8.78	8.10	7.89	7.88	
16	8.22	8.20	8.56	8.85	8.85	calibr error	8.87	8.88	7.96	7.95	7.96	
17	8.40	8.32	8.56	8.91	8.92	calibr error	8.99	9.19	8.04	7.99	8.38	
18	8.43	8.32	8.54	8.82	8.90	calibr error	8.89	8.94	8.12	7.89	8.61	
19				8.38	8.44	calibr error	8.31	8.55	7.44	8.20	8.54	

Averages												
A / Urine	8.33	8.28	8.60	8.93	8.90	9.02	8.84	8.92	8.13	7.90	7.91	
A / Effluent	8.47	8.35	8.45	8.70	8.81		8.84	8.97	8.14	7.90	8.24	
A / E + DAP	8.14	8.16	8.63	8.95	8.77	9.00	8.73	8.84	8.01	7.92	7.96	
S / Urine	8.29	8.25	8.67	8.98	8.85	9.01	8.79	9.17	8.12	8.03	8.41	
S / Effluent	8.34	8.30	8.56	8.83	8.80	8.98	8.72	9.07	8.12	8.01	8.47	
S / E + DAP	8.26	8.25	8.56	8.72	8.81	9.18	8.83	9.06	8.00	8.07	8.56	

Following page:

Appendix H: Poster

Nutrient Recovery from Urine and Struvite Production Effluent Using Aquatic Plants in Nepal

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Bachelor Thesis in Environmental Engineering, Aug. 2010
Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

Introduction

Urine is a valuable resource that can be reused in agriculture, curbing both the need to buy expensive commercial fertilizers and at the same time preventing eutrophication of water bodies through uncontrolled sewage discharge. However, direct field application of liquid urine is limited due to storage, transportation, and socio-cultural constraints.

The precipitation of struvite ($MgNH_4PO_4$-$6H_2O$) is an option to trap almost all the phosphorus (P) from urine as a solid, storable and easily applicable slow-release fertilizer. Aquaculture may be another possibility to recover the remaining nutrients from struvite production effluent – mainly nitrogen (N) and potassium (K) – or from urine itself, leading to protein-rich plant biomass that can be used as green manure or animal feed.

The objective of this study was to assess the suitability of aquatic plants for nutrient removal and biomass production in the Nepalese context. The experimental site was located in the village of Siddhipur in the Kathmandu Valley (Fig. 1).

Fig. 1: Experimental site in Siddhipur, Nepal

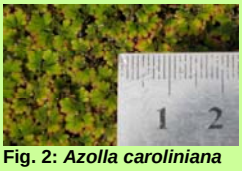

Fig. 2: *Azolla caroliniana*

Fig. 3: *Spirodela polyrrhiza*

Methodology

The floating macrophytes *Azolla caroliniana* (Fig. 2) and *Spirodela polyrrhiza* (Fig. 3) were selected and grown in 35-L tanks with diluted urine, effluent, and a control treatment with effluent and added diammonium phosphate (DAP) to resolve if P was the growth-limiting factor (3 replications per treatment).

Over a 22-day period, photospectrometric analyses of growing medium samples determined removal of ammonium (NH_4-N), phosphate (PO_4-P), and potassium (K). The tanks were re-fertilized weekly to initial levels of 20mg·L^{-1} NH_4-N. At the end of the trial, biomass measurements assessed dry matter increase as well as total N content of *Spirodela*.

Results & Discussion

Biomass production

Azolla was easily available and produced more biomass than *Spirodela* in all growing media, probably also thanks to higher inoculation density. Better results for *Spirodela* might be achievable in shaded ponds and with higher initial coverage to prevent competition from algae. A restricting factor is the limited availability of *Spirodela* inoculum in the winter months.

The tanks with added DAP showed lower biomass production but healthier plants and less algae. In *Azolla* grown on effluent increasing signs of P deficiency became apparent (red coloration).

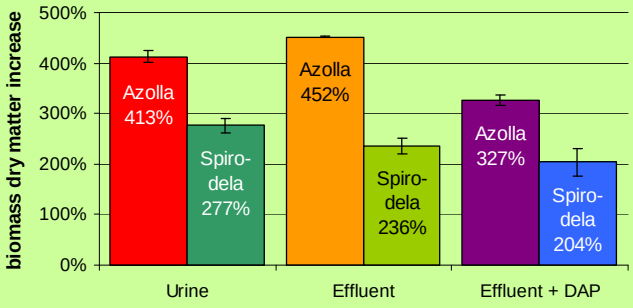

Fig.4: Biomass: % increase of inoculum in terms of dry matter

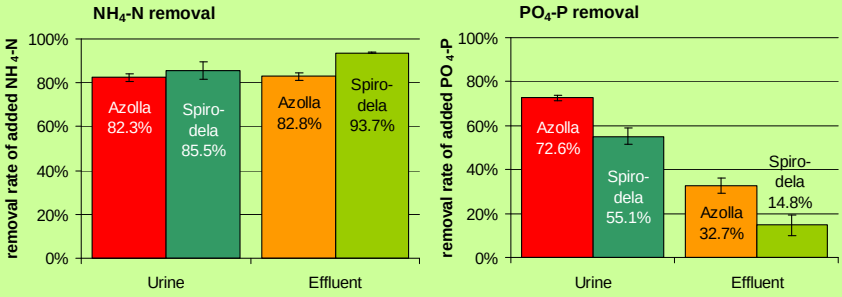

Fig. 5: Recorded removal rates for NH_4-N (left) and PO_4-P (right)

Nutrient removal

NH_4-N was removed more efficiently from *Spirodela* tanks while *Azolla* tanks achieved higher removal rates for PO_4-P (Fig. 5). K analysis allowed no substantive interpretation (low sample size).

The ratio of N assimilation by *Spirodela* was only marginal with 1.05-2.80% of total N removal. It must be assumed that most N was lost through other processes such as denitrification and volatilization of ammonia (NH_3).

Conclusion

Nutrient removal from urine with *Azolla* and *Spirodela* under the climatic conditions of early spring in Kathmandu is possible. Effluent as a growing medium can only be recommended for short term treatment: Even though higher NH_4-N removal rates and – for *Azolla* – more biomass production were recorded, P deficiency is expected to inhibit plant growth in the long run.

Further research needs

- quantification of contribution of plants towards nutrient removal
- year-round production feasibility
- assessment of need for and use of produced biomass
- economic viability of nutrient recovery through *Azolla* and *Spirodela* in Nepal

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