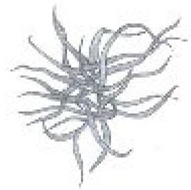
DUCKWEED FORUM

Newsletter for the Community of Duckweed Research and Applications



Vol. 4(1): 204 – 237 (2016), part #12

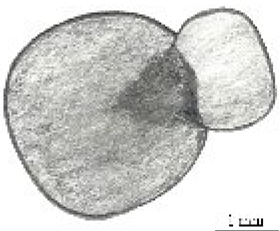
Edited by the International Steering Committee on Duckweed Research and Applications

Water buffalo enjoying *Wolffia microscopica* in Varanasi, Uttar Pradesh, India near the holy river Ganga. Photo: Klaus-J. Appenroth, University of Jena, Germany.

International Steering Committee on Duckweed Research and Applications Members:

Information about ISCDRA and prior issues are available at http://lemnapedia.org/wiki/ISCDRA

Science meets Art



Wolffiella hyalina (Delile) Monod is one of the ten rootless species of the genus *Wolffiella*. It exists in rather dry climates of Africa from the South to the North. However, in the recent years, KS Sree and KJ Appenroth found it also around Hyderabad, Telangana, India where it has been evidently introduced – as already stated by E. Landolt some decades back. Drawing: Dr. K. Sowjanya Sree, Amity University Uttar Pradesh, India.

Letter from the editor

Dear Friends of Duckweed,

The New Year has started and we begin with this issue the fourth year of our Newsletter, now called "Duckweed Forum" (DF). We wish you all a HAPPY NEW YEAR in your personal life and in duckweed affairs.

It is our ambition to offer you again four times this year with DF issues that will be as colourful and interesting as possible. For the present issue, in the section "Science meets Art", Sowjanya Sree presents again drawing of a species from the genus *Wolffiella*. Evan Ernst from Cold Spring Harbour Lab reports about progress in their genome sequencing of the species *Lemna minor* and *Lemna gibba*, and in our sequel "Student Researcher Spotlight" Paul Fourounjian from Rutgers University of New Jersey (Lab of Jo Messing) is introduced. Louis Landesman, member of the ISCDRA, reports about his duckweed excursion to Indonesia and Ron Salpeter introduces the duckweed producing company "Hinoman" from Israel. For duckweed-related information that may be of your interest, you will find new installments in our sections "Useful Methods" (about stock cultivation of duckweed) and "Experiments for students" (about chlorophyll fluorescence). As always we finish our DF with the newest literature from our database provided together with corresponding abstracts for the original articles.

We are happy to inform you that a summary of our 3rd International Conference on Duckweed Research and Applications in Kyoto 2015 was published in the December issue of "Plant Molecular Biology". We are very grateful to the editor that they managed the publication so fast and even used a duckweed photo as the cover for this issue stressing this report: Klaus-J. Appenroth, K. Sowjanya Sree, Tamra Fakhoorian, Eric Lam. Resurgence of duckweed research and applications: report from the 3rd International Duckweed Conference. Plant Molecular Biology 89(6): 647-654 (2015)

If you do not have access to this article, please, ask the corresponding author Eric Lam, member of the ISCDRA. It can also be downloaded from the RDSC site as well.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),
Klaus-J. Appenroth, Head

Human Grade Duckweed Cultivation by Hinoman

Hinoman's duckweed, known as "Mankai" is produced in a covered outdoor hydroponics system that optimizes growth. The proprietary cultivation algorithm, along with other technological elements, is geared toward human grade consumable duckweed. Hinoman's hydro-culture process consumes a minimum of water and energy, while leaving a negligible ecological footprint. The company is currently focused on cultivation methods that will enable it to grow and adapt the duckweed to a variety of climates and geographical locations. The methodology pays special attention to a long list of parameters affecting the growth, such as temperature, humidity, light and others. The company is fortunate to have a number of world class consultants working very closely with it since several years, including for example Prof. Marvin Edelman – one of the Duckweed community's most esteemed scientists.

Dr. Klaus Appenroth, Prof. Marvin Edelman and Ron Salpeter (Hinoman's CEO)

Multiple research disciplines are at the core of Hinoman's cultivation methodology, and are designated to enable human grade consumable plant; this includes close monitoring of the plant with data collected into a "Mankai database" which permits comprehensive understanding of the plant.

Mankai is a non-GMO strain, yet a bred strain, that has been cultivated for close to a decade. Its growth media does not use pesticides, fungicides or growth stimulators (including hormones).

Mankai is a high protein, vitamin and mineral rich unprocessed source, all being natural.

Its composition has great nutritional benefits by containing 45%+ protein on a dry weight basis, a superior vitamin profile as well as minerals such as calcium, iron, zinc, magnesium and low sodium and low sugar (Fig.1).

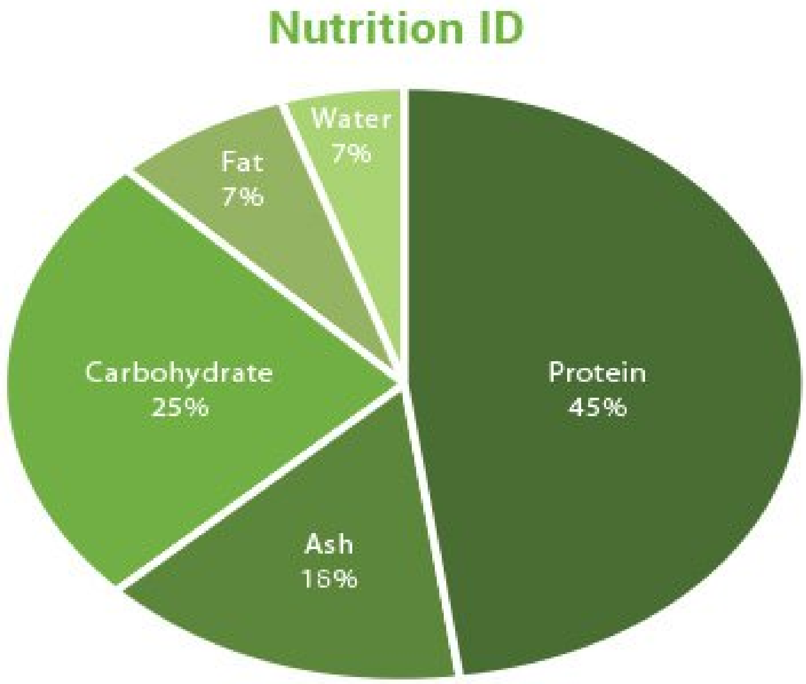


Fig.1. Hinoman's cultivation methodology produces a low sodium low sugar Mankai.

This is a nutritional profile, superior in many ways to soy, kale or spirulina, (Fig. 2). Mankai is a source of beneficial amino acids as its essential amino acid content is very close to that of ideal protein for humans as recommended by FAO/WHO. Its specific amino acids content confer benefits to human health during period of physical effort, illness or stress.

Mankai has a neutral, mild taste, as opposed to green aqua-cultured algae, and can be used fresh or dry as a food supplement or food ingredient, with a wide range of food and beverage applications.

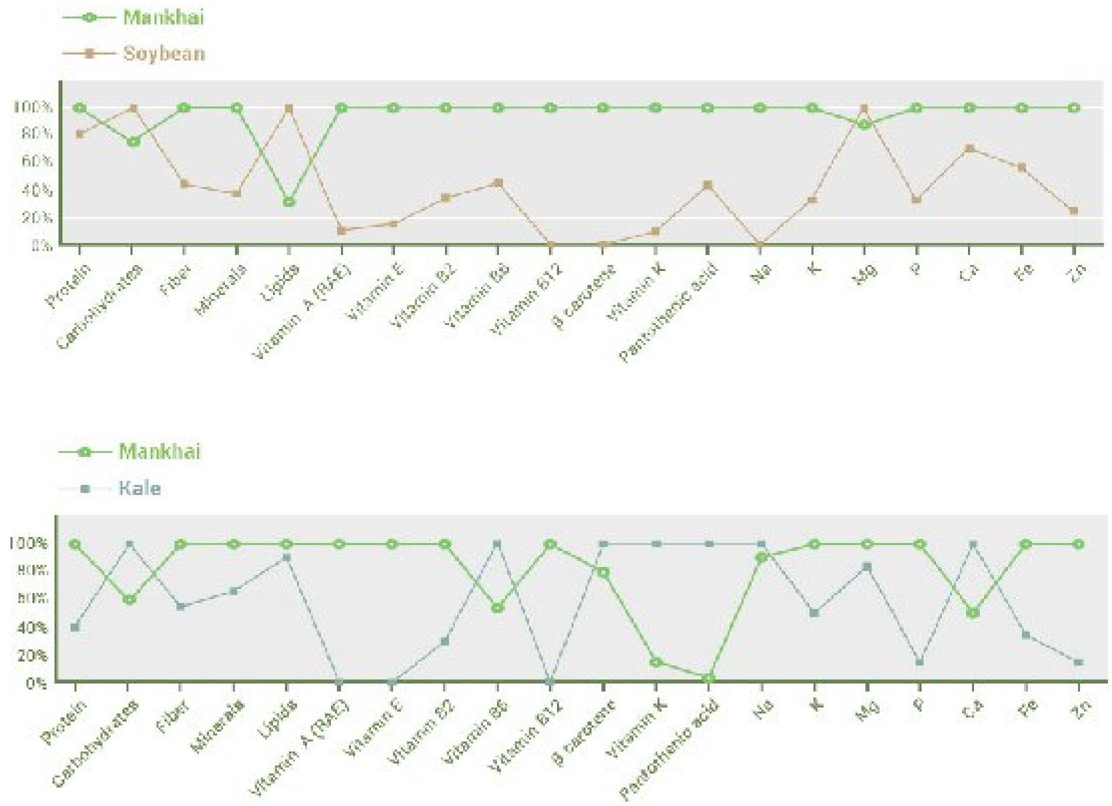


Fig.2 Comparative nutritional profiles. Percent values prorated to the higher between the two compared vegetables. Source: USDA.

Status of the Lemna gibba 7742a and Lemna minor 8627 genomes

Evan Ernst
Martienssen Lab
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In 2009, Cold Spring Harbor Laboratory hosted a meeting funded by the Moore Foundation to advance interest and involvement in the biology and genetics of aquatic plants. Subsequently, we began a collaboration with researchers at Brookhaven National Laboratory and Stonybrook University that produced a survey of lipid production diversity in 30 duckweed species[1] and established crucial tools for reengineering lipid metabolism in *Lemnaceae*: a rapid protocol for stable genetic transformation, an artificial miRNA knockdown approach[2], and draft genome sequences of *Lemna gibba* 7742a (G3) and *Lemna minor* 8627.

The initial draft of the ~450 Mbp *L. gibba* 7742a genome was highly fragmented and unwieldy, but today the assembly is of comparable quality to the *Musa acuminata* reference genome at the contig level. In the absence of a physical or genetic map, the scaffolds are not organized into pseudomolecules, though half of the assembly is contained in scaffolds longer than 0.52 Mbp, and in contigs longer than 53 Kbp. The most recent annotation contains 21,830 protein-coding gene loci.

We presented our first draft of the *L. minor* 8627 genome at the ICDRA in Kyoto this year. Since then we have made significant improvements to the quality of the assembly. Incorporating additional coverage by PacBio single molecule long reads enabled primary contig assembly solely from this data type, which does not suffer from the nucleotide composition dependent coverage biases induced by technologies that rely on DNA amplification. Consequently, the contig number was reduced from nearly 40,000 to fewer than 9,000 and the contig N50 increased from 65 Kbp to 222 Kbp. These longer contigs better resolve repetitive and low-complexity regions flanking genes that can impact gene regulation.

Using flow cytometry, we estimated the *L. minor* 8627 1C genome size to be 683 Mbp, close to the 763 Mpb total span of the new assembly. Overassembly of diploid and highler ploidy genomes can occur due to regions of high heterozygosity failing to collapse in the haploid assembly. This estimate is larger than any previously reported for *L. minor* (the second largest being 604 Mbp for clone 7136), and nearly twice the size of the clones with the smallest genomes[3]. Considering the two-fold greater number of protein coding gene predictions in this clone compared to *S. polyrhiza* 7498[4], *L. gibba*, 7742a and the just published *L. minor* 5500[5] genomes, we conclude that *L. minor* 8627 has undergone a recent whole genome duplication.

Rapid, inexpensive sequencing and near-perfect assembly of even the most expansive and repetitive genomes is on the horizon. Octoploidy and genome sizes close to 2 Gbp have been observed in *Wolffia*[3], traits that pose significant challenges. Yet advances in single molecule sequencing will soon make them tractable due to decreasing cost per base covered and average read lengths approaching fosmid insert sizes that can span multiple polymorphisms between homeologous chromosomes.

We appreciate the feedback we've received from many who are eager to get to work with these two genomes ahead of publication. The latest draft assemblies are available at www.lemna.org under a data usage policy consistent with the Fort Lauderdale Agreement.

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Duckweed in Indonesia

By Dr. Louis Landesman

Last March, April, October and November 2015 I visited Indonesia to advise the Indonesian NGO Yayasan Rumah Energi on incorporating duckweed in integrated farming systems. Rumah Energi promotes the use of duckweed grown on bioslurry, the liquid effluent from biodigesters (anaerobic digesters) that use manure from cattle as their raw material for digestion. Duckweed grown on bioslurry would then be used to feed to fish, chickens or dairy cattle living on the farm.

Due to drought conditions in Indonesia I collected fresh duckweed from the Bogor Botanical gardens in Bogor south of Jakarta for restocking in Lembang.

Duckweed at Bogor Botanical Garden (Kebun Raya)

I visited farms near Jogjakarta and Bandung and helped set up a small experimental facility on a cooperative demonstration dairy farm in Lembang, a small town north of Bandung in West Java.

Duckweed facility in Java

Duckweed harvesting

Weighing Duckweed

I also visited Vietnam again in October 2015 and met a farmer planning to use duckweed to feed his pigs.

Duckweed pond in Vietnam

Farrowing Unit

Pig enclosure

Useful methods 4: Stock cultivation of duckweed

K. Sowjanya Sree[1] and Klaus-J. Appenroth[2]
[1]Amity Institute of Biotechnology, Amity University UP, Noida, India
[2]University of Jena, Plant Physiology, Dornburger Str. 159, 07743 Jena, Germany

In the previous issue of "Duckweed Forum" (DF 3, 180-186 (2015)) we described several media standardized for duckweed cultivation. These are liquid media used for optimal cultivation of duckweeds at high growth rates. For long-term cultivation such high growth rates are not desired because aliquots of the cultures would have to be transferred into fresh nutrient medium between 2 weeks and perhaps 2 months, depending on clone and species. Cultivation of stocks thus means, at first, to reduce the rate of growth.

The first measure is to reduce the temperature. The optimal growth rates are measured under standardized temperature, at 25°C (DF 3, 59-62 (2015)). For stock cultivation we reduce the temperature to 18°C. All the duckweed species can deal with this temperature, of course with a dramatically reduced growth rate. Even 15°C is possible. The late Elias Landolt mentioned that clones collected from tropical climates cannot tolerate 5°C for a longer time.

The next important environmental factor is the light intensity. The light intensity is reduced from 100 µmol m-2 s-1 continuous white light (standardized for optimal growth rate) to ca. 30 µmol m-2 s-1. We observed that duckweeds are able to deal with low light intensity only when the temperature is also reduced. At 25°C several species (e.g. from the genus *Wolffiella*) die at such low light intensities. This light intensity seems to be close to the photon compensation point.

It is also very useful to reduce the water availability. This can be done by replacing the liquid medium by Agar based medium. Instead of Agar also a kind of synthetic Agar, GELRITE, can be used. These semisolid media have to be prepared with a defined nutrient medium to supply the macro and micro nutrients to plants. We normally use N-medium (DF 3, 182-183 (2015)). The concentration of KH2PO4 is increased to 1 mM. At the low concentration used in liquid medium (e.g. 60 µM), the survival of plants on Agar would be for a very short duration. However, also other media are possible. Often MS-medium is used because this medium is commercially available as a ready-to-use mixture.

Solid Agar is added to nutrient medium, normally at a concentration of 0.9 %. For sensitive clones, addition of as low as 0.7 % Agar is possible. Gelrite is used at a concentration of ca. 0.45 %. Normally one litre Agar suspension is prepared, heated it in a microwave oven to 80°C and 50 – 75 ml of it is poured into each of the 100 ml-Erlenmeyer flask secured with cotton wool stoppers. The flasks are then autoclaved. It is indeed a very large amount of Agar in each flask having the advantage that the life time of stock cultures is very long ca. 4 to 5 or even 6 months. In other stock collections standard glass test tubes are used, filled with 5 ml Agar medium and also closed with cotton wool stoppers. Alternatively, sterile plastic Petri dishes could also be used. The diameter of the Petri dishes might be 9 cm (standard quality, very cheap) or much smaller, down to a diameter of 3 cm in order to spare medium and space. In these cases, the Agar medium is first autoclaved, normally in 1 L flasks, and then poured into sterile Petri dishes in a laminar flow box. After inoculating the plants, the Petri dishes are closed by parafilm to prevent faster rate of drying of the Agar layer.

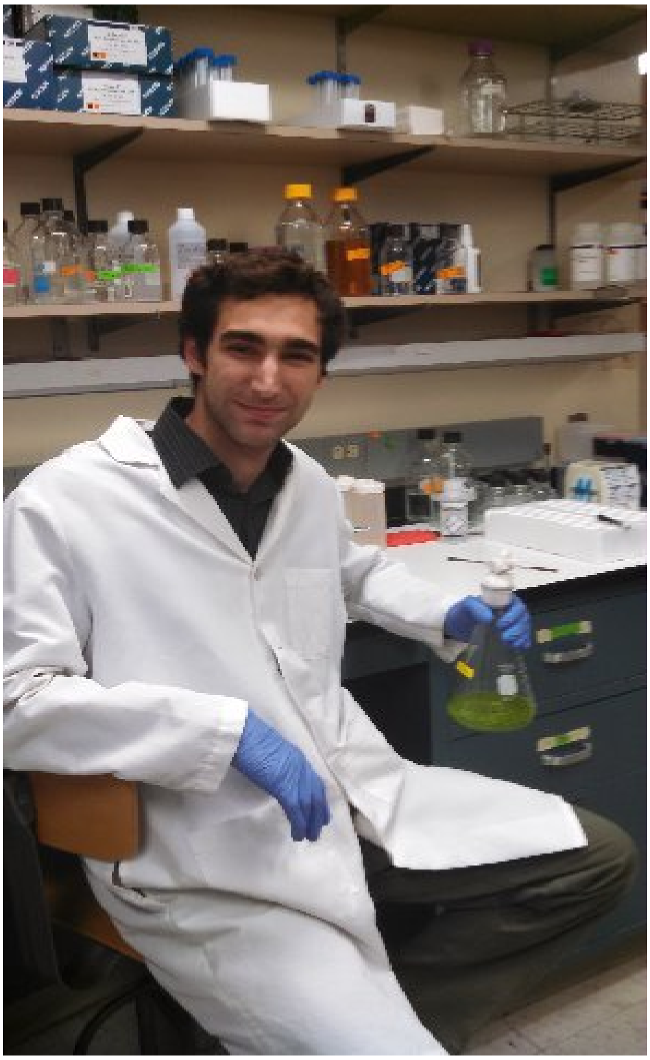
In order to control the sterility (in terms of pure, uncontaminated cultures) of plants it is useful to add low molecular weight carbohydrates. Glucose (50 mM) or sucrose (25 mM) are commonly used. We learned recently, that in some cases (e.g. *Wolffia*) after sterilization, 50 mM glucose is too high for their regeneration and plants often die. In this case, but perhaps also in general, 25 mM glucose or 25 mM sucrose are better used in Agar or gelrite media. Fructose is not recommended.

Species of the genus *Spirodela* (*S. polyrhiza*, *S. intermedia*) tend to grow in several layers, one above the other. As a consequence, the younger fronds in the upper most layers lose their contact to water supplying Agar and die thereafter. Therefore, for these two species, we use liquid medium without sugar. The disadvantage is that the contaminations cannot be recognized and in each case a sterility test is necessary before using these plants. When sugar is added, the survival of the fronds is too short.

For *S. polyrhiza* there exists another opportunity for their long-term preservation. This species forms turions, survival organs that sink to the bottom of the flasks and can easily be harvested. Turions can be stored in a fridge (5°C, darkness) for several years. Turion formation can be easily initiated by inoculating plants in a liquid medium with low phosphate concentrations, e.g. 60 µM. The Inoculated plants and their emerging daughters use the available phosphate for vegetative growth and after a certain time period, the drastically reduced phosphate concentration(e.g. to 2 µM) induces turion formation. Addition of glucose (50 mM) is recommended to accelerate this process and to increase the turion yield.

Student Spotlight - Paul Fourounjian

Paul is a microbiology and molecular genetics doctoral fellow at Rutgers University



As a child and teen, I was interested in sustainable energy, wanting to know about our large-scale infrastructure: Where does our electricity come from? Where does our sewage and garbage go? How can we improve these services? Finally, how much energy could we save if we made them even 1% more efficient? So, when I graduated from The College of New Jersey in 2012 with a Bachelors degree in Biology, and a few engineering classes, I was looking forward to a career of developing microalgae as a feedstock for biodiesel. During my graduate school search, I had an interview with Dr. Messing from Rutgers University. He explained a beautifully simple process to create bioenergy out of a rapidly growing plant that avoided the engineering challenges of harvesting algae and extracting their oil. That was when I chose duckweed genetics and applications for my thesis and hopeful career path.

Since the 2014 publishing of the *Spirodela polyrhiza* genome sequence, as a reference for the family, we're trying to see how these genes work under 8 different conditions and how they are regulated by microRNAs (miRNAs). With small RNA sequencing and prediction software, we, along with our collaborators, are able to predict the miRNAs and their mRNA targets. We're also using a new method called Degradome Sequencing to identify the cleaved mRNA fragments and confirm the predicted miRNAs cleavage activity on the predicted mRNA targets. The addition of seven stress and hormonal conditions lets us see which miRNAs and their targets are regulated in response to each stimuli. This should result in the first catalog of miRNAs found in *Spirodela polyrhiza*, their targets, and their expression profiles to further understand the growth of duckweeds at the molecular level.

Together with our collaborators we hope that this dataset will further the understanding of gene expression and regulation of these species and help to guide future research and applications. Hopefully many scientists will look at this data and make their own important discoveries, whether it's learning more about the effect of copper on aquatic plants, seeing what the impacts of high nitrate availability are, or overexpressing or suppressing a miRNA to keep part of the sugar response or cold stress response pathway active. In this way, we hope to augment the foundation for the growing duckweed research community.

The Messing lab works on a wide range of genomic and applied projects in maize, sorghum, and duckweeds. My labmates work with genomic and transcriptomic research, investigating the fundamental science of maize development alongside projects such as developing high sugar sorghum and sugar cane varieties, successfully baking bread from gluten-free corn, and methionine rich corn, designed to eliminate the need of methionine supplementation in animal feed. Dr. Wenqin Wang, currently a professor at Shanghai Jiao Tong University, was a former student and post-doctoral researcher in the Messing lab. She was my mentor in duckweed research. She instilled in me an appreciation of bioinformatics and taught me to tackle complicated problems with a smile.

In July I had the pleasure and privilege to attend the 3rd ICDRA in Kyoto. It was a great chance to share my current project, learn about all the exciting fields of research, and bond with all the researchers behind this progress. My friends study diverse mature topics, and they are all envious of the duckweed research community for the way we see ourselves as a global team working to improve the world while helping ourselves.

To stay involved in the practical aspects of duckweed research, and that research community, I have been an active member of the International Lemna Association, which I recommend to everyone reading, especially to my fellow students. It taught me about the applications of duckweeds, and their integration with topics as diverse as governmental regulation, ethanol fermentation, and human nutrition. By actively participating in the ILA, I learned so much more about the industry I will enter, and even as a student, occasionally am able to help the people who are growing and using tons of duckweed.

Experiments for students 3: Chlorophyll fluorescence in vivo

Klaus-J. Appenroth
University of Jena, Plant Physiology, Dornburger Str. 159, 07743 Jena, Germany

Isolating chlorophyll and carotenoids from plant tissues is very often carried out in basic courses of Plant Physiology. Beside determining the concentration by absorbance measurements in a spectrometer or separating the different compounds by thin layer chromatography, chlorophyll fluorescence can be investigated. After homogenization of green plant tissue with a suitable organic solvent and perhaps after filtration or centrifugation, fluorescence can be observed directly under a UV-B lamp. This can be done with practically all green plants. Duckweed offer an additional opportunity. As the cuticula is almost non-existent, substances are therefore easily taken up from liquid medium. This holds true e.g. for herbicides that uncouple the photosynthetic electron transport. Then, the energy cannot be transported away from photosystem II and the probability for fluorescence is increased. Theoretically, this can be investigated by pulse-amplitude modulated fluorescence (PAM) or by direct fluorescence measurements (OJIP method). However, equipment available for that are normally not available for student courses.

We used the following experiment to demonstrate inhibition of the photosynthetic electron transport in duckweed, e.g. *Lemna minor*:

Fronds of duckweed were put in a petri dish and a few droplets of a solution of DCMU ((3-(3,4-dichlorophenyl)-1,1-dimethylurea; Diuron) on the surface is applied. Using a UV-B lamp (eye protecting specs are required) the fronds are irradiated. Whereas untreated areas show no fluorescence, the treated area shows red fluorescence after a few minutes. The lack of this effect when other leafs are used (e.g. garden bean) demonstrates the importance of the cuticula for terrestrial plants in contrast to water plants.

DCMU solution: the final concentration should be 1 mM (molecular mass 233 g mol^{-1}). The solid substance is pre-solved with as less as possible acetone. Then the solution is diluted to 1 mM with water.

Mechanism: The herbicide DCMU blocks the plastoquinone binding site of photosystem II interrupting this way the photosynthetic electron transport from PSII to PSI. As a consequence fluorescence can be observed even with naked eye.

From the Database

Highlights

Resurgence of duckweed research and applications: report from the 3rd International Duckweed Conference

Appenroth, K-J; Sree, KS; Fakhoorian, T; Lam, E

Duckweed, flowering plants in the Lemnaceae family, comprises the smallest angiosperms in the plant kingdom. They have some of the fastest biomass accumulation rates reported to date for plants and have the demonstrated ability to thrive on wastewater rich in dissolved organic compounds and thus could help to remediated polluted water resources and prevents eutrophication. With a high quality genome sequence now available and increased commercial interest worldwide to develop duckweed biomass for renewables such as protein and fuel, the 3rd International Duckweed Conference convened at Kyoto, Japan, in July of 2015, to update the community of duckweed researchers and developers on the progress in the field. In addition to sharing results and ideas, the conference also provided ample opportunities for new-comers as well as established workers in the field to network and create new aliances. We hope this meeting summary will also help to disseminate the key advances and observations that have been presented in this conference to the broader plant biology community in order to encourage increased cross-fertilization of ideas and technologies.

Transformation of Wolffia arrhiza (L.) Horkel ex Wimm

Khvatkov, P; Chernobrovkina, M; Okuneva, A; Pushin, A; Dolgov, S

PLANT CELL TISSUE and ORGAN CULTURE 123: 299-307 (2015)

To date, *Lemna* and *Spirodela* species of the family Lemnaceae are the only species that have been used to produce recombinant proteins for pharmaceutical and veterinary purposes. *Wolffia arrhiza* is the most evolutionarily advanced species of the Lemnaceae. A rootless duckweed, it is the most promising target for biopharming as a candidate for submerged cultivation in a fermenter. As a first step toward future biotechnological use of *Wolffia*, we established a stable transformation system for it based on Agrobacterium-mediated transformation. Following inoculation with the bacteria, *Wolffia* cluster explants were cultured for 2 weeks on media containing 2.0 mg l(-1) 2,4-dichlorophenoxyacetic acid and 2.0 mg l(-1) N-6-benzyladenine. Explants were then transferred to growth regulator-free media in the presence of 5.0 mg l(-1) hygromycin B to select antibiotic-resistant plants. Other selective agents-kanamycin and phosphinothricin-were not suitable for *Wolffia*, nor was application of particle bombardment for the delivery of foreign DNA to *Wolffia* explants. The developed agro transformation conditions yielded stably transformed lines of *Wolffia*, confirmed by Southern blotting, with an efficiency of 0.2-0.4 % transgenes per 100 explants.

Biotechnology

Duckweed biomass as a renewable biorefinery feedstock: Ethanol and succinate production from Wolffia globosa

Soda, S; Ohchi, T; Piradee, J; Takai, Y; Ike, M

BIOMASS and BIOENERGY 81: 364-368 (2015)

For evaluating duckweed biomass as a bioresource, the specific growth rate and the chemical constituents of duckweed of four kinds were investigated. *Spirodela polyrrhiza*, *Lemna minor*, *Wolffia arrhiza*, and *Wolffia globosa* commonly showed high specific growth rates of 0.22-0.30 d(-1) with initial concentrations of nitrogen >3.0 kg m(-3) and phosphorus >5.0 kg m(-3). All duckweeds had high sugar contents greater than 300 g kg(-1) of dry mass. Especially, vegetative fronds of *W. globosa* showed the highest sugar content of 410 g kg(-1) of dry mass. The duckweed biomass was pretreated easily by heating at 121 degrees C for saccharification using a-amylase and amyloglucosidase. The ethanol yield of *W. globosa* biomass in the simultaneous saccharification and fermentation (SSF) using the enzymes and dry yeast was 170 g kg(-1) of dry mass, whereas the succinate yield in the SSF using the enzymes and *Actinobacillus succinogenes* was 200 g kg(-1) of dry mass. The production rates of ethanol and succinate from the *W. globosa* biomass were estimated as 0.58 kg m(-2) y(-1) and 0.68 kg m(2) y(-1), respectively. The biomass of duckweed, with its high growth rate and high starch content, can be an excellent renewable feedstock for the production of ethanol and succinate as building block chemicals for the replacement of petrochemicals.

Using proteomic analysis to investigate uniconazole-induced phytohormone variation and starch accumulation in duckweed (Landoltia punctata)

Huang, MJ; Fang, Y; Liu, Y; Jin, YL; Sun, JL; Tao, X; Ma, XR; He, KZ; Zhao, H

BMC BIOTECHNOLOGY 15, Article Number: 81, DOI: 10.1186/s12896-015-0198-9 (2015)

Duckweed (*Landoltia punctata*) has the potential to remediate wastewater and accumulate enormous amounts of starch for bioethanol production. Using systematical screening, we determined that the highest biomass and starch percentage of duckweed was obtained after uniconazole application. Uniconazole contributes to starch accumulation of duckweed, but the molecular mechanism is still unclear. To elucidate the mechanisms of high starch accumulation, in the study, the responses of L. punctata to uniconazole were investigated using a quantitative proteomic approach combined with physiological and biochemical analysis. A total of 3327 proteins were identified. Among these identified proteins, a large number of enzymes involved in endogenous hormone synthetic and starch metabolic pathways were affected. Notably, most of the enzymes involved in abscisic acid (ABA) biosynthesis showed up-regulated expression, which was consistent with the content variation. The increased endogenous ABA may up-regulate expression of ADP-glucose pyrophosphorylase to promote starch biosynthesis. Importantly, the expression levels of several key enzymes in the starch biosynthetic pathway were up-regulated, which supported the enzymatic assay results and may explain why there is increased starch accumulation. These generated data linked uniconazole with changes in expression of enzymes involved in hormone biosynthesis and starch metabolic pathways and elucidated the effect of hormones on starch accumulation. Thus, this study not only provided insights into the molecular mechanisms of uniconazole-induced hormone variation and starch accumulation but also highlighted the potential for duckweed to be feedstock for biofuel as well as for sewage treatment.

Enriching duckweed as an energy crop for producing biobutanol using enzyme hydrolysis pretreatments and strengthening fermentation process using pH-stat

Su, HF; Xu, GH; Chen, HZ; Xu, YJ

ACS SUSTAINABLE CHEMISTRY and ENGINEERING 3: 2002-2011 (2015)

Large scale production of biobutanol from a lignocellulosic feedstock for alternative fossil fuels consumption has garnered much interest by researchers in renewable energy. However, making biobutanol from lignocellulose requires the development of novel, renewable, nonfood sources for biofuel production and sustainable biorefining technology that maximizes the utilization of feedstock is indispensable. Duckweed (Lemnaceae) is a family of aquatic plants that in early trials has demonstrated great potential as an alternative nonfood energy feedstock for ethanol production. However, research on methods to obtain higher biobutanol yield from this plant is thus far insufficient. In this study, we tested several hydrolysis procedures with different enzyme combinations for duckweed pretreatment in detail. We then assessed the efficiency of these treatments for biobutanol production via fermentation with *Clostridium acetobutylicum*, using separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF) and modulation of pH with pH-stat. The highest concentration of butanol and total solvent produced via SHF were 11.63 g/L and 24.06 g/L, respectively, using an enzyme hydrolysis method 4 (EHM4) with pH control. With SSF and controlled pH, butanol and total solvent concentrations achieved by EHM4 were 13.56 and 26.78 g/L, respectively, which was 14% and 10% higher than with SHF. Our results also show that duckweed is a promising feedstock for biobutanol production via comparison experiments. This study shows an additional advantage of using duckweed as a fermentation substrate is the potential to use simple enzyme hydrolysis instead of complex pretreatment. Having demonstrated the greatest butanol yield thus far, this study indicates that duckweed is a very promising bioenergy crop for industrial biobutanol development.

Ecology

The effect of floating vegetation on CH4 and N2O emissions from subtropical paddy fields in China

Wang, C; Li, SC; Lai, DYF; Wang, WQ; Ma, YY

PADDY and WATER ENVIRONMENT 13: 425-431 (2015)

Duckweed (*Lemna minor*), a floating macrophyte belonging to the Lemnaceae family, is commonly found in subtropical paddy fields. This plant rapidly takes up nutrients from water and forms dense floating mats over the water surface that may impact the biogeochemical processes and greenhouse gas production in paddy fields. In this study, we measured CH4 and N2O emissions from duckweed and non-duckweed plots in a subtropical paddy field in China during the period of rice growth using static chamber and gas chromatography methods. Our results showed that CH4 emission rate ranged from 0.19 to 26.50 mg m(-2) h(-1) in the duckweed plots, and from 1.02 to 28.02 mg m(-2) h(-1) in the non-duckweed plots. The CH4 emission peak occurred about 1 week earlier in the duckweed plots compared to the non-duckweed counterparts. The mean CH4 emission rate in the duckweed plots (9.28 mg m(-2) h(-1)) was significantly lower than that in non-duckweed plots (11.66 mg m(-2) h(-1)) (p < 0.05), which might be attributed to the higher water and soil Eh in the former. N2O emission rates varied between -50.11 and 201.82 A mu g m(-2) h(-1), and between -28.93 and 54.42 A mu g m(-2) h(-1) in the duckweed and non-duckweed plots, respectively. The average N2O emission rate was significantly higher in the duckweed plots than in the non-duckweed plots (40.29 vs. 11.93 A mu g m(-2) h(-1)) (p < 0.05). Our results suggest that the presence of duckweed will reduce CH4 emission, but increase N2O flux simultaneously. Taking into account the combined global warming potentials of CH4 and N2O, we found that growing duckweed could reduce the overall greenhouse effect of subtropical paddy fields by about 17 %.

Feed and Food

Water lentils (duckweed) in Jordan irrigation ponds as a natural water bioremediation agent and protein source for broilers

Shammout, MW; Zakaria, H

ECOLOGICAL ENGINEERING 83: 71-77 (2015)

The growth of wild water lentils (duckweed) in Jordan's farm irrigation ponds has focused attention on the need to study the functions of these plants as a natural water bioremediation agent and as a potential protein source for broilers. This paper examined the water source and water quality (nutrient composition) of irrigation ponds that support duckweed plants and their suitability as broilers feed. The results showed that the source of the irrigation ponds is Zarqa River, and the analyzed water quality parameters of the source fall within the allowed limits set forth in the Jordanian Standard. The growth of duckweed in farm irrigation ponds reduces the nutrient supply to irrigated crops and serves as a purifier of irrigation water. These plants remove an average of 20% Ca, 33% Mg, 21% K, 13% Cl, 25% SO4, 35% PO4, 1.5% Na, and 40% NO3. Fresh duckweed contains approximately 94% water and 6% dry matter; the average nutrient concentrations in dry matter are 26% protein, 4.16 % N, 5.2% fiber, 3.1% fat, 0.86% P, 2.4% K, 4.3% Ca, 0.88% Mg, 0.2% Fe, 1.62% Cl, 0.16% Na, 0.008% Zn, 0.07% Mn and 0.002% Cu. This research reveals the potential for Jordan to examine the availability of duckweed in farm irrigation ponds and their function as water bioremediation agents and their suitability as a protein and nutrient source for broilers. If harvested at frequent intervals and dried, water lentil plant (duckweed) could be of great benefit to Jordan's livestock sector, especially with regard to poultry, where feedstuffs are limited in supply and prices are typically high.

Molecular Biology

The map-based genome sequence of Spirodela polyrhiza aligned with its chromosomes, a reference for karyotype evolution

Cao, HX; Vu, GTH; Wang, W; Appenroth, KJ; Messing, J; Schubert, I

The New Phytologist 209: 354-63 (2016)

Duckweeds are aquatic monocotyledonous plants of potential economic interest with fast vegetative propagation, comprising 37 species with variable genome sizes (0.158-1.88Gbp). The genomic sequence of *Spirodela polyrhiza*, the smallest and the most ancient duckweed genome, needs to be aligned to its chromosomes as a reference and prerequisite to study the genome and karyotype evolution of other duckweed species. We selected physically mapped bacterial artificial chromosomes

(BACs) containing *Spirodela* DNA inserts with little or no repetitive elements as probes for multicolor fluorescence in situ hybridization (mcFISH), using an optimized BAC pooling strategy, to validate its physical map and correlate it with its chromosome complement. By consecutive mcFISH analyses, we assigned the originally assembled 32 pseudomolecules (supercontigs) of the genomic sequences to the 20 chromosomes of *S.polyrhiza*. A *Spirodela* cytogenetic map containing 96 BAC markers with an average distance of 0.89Mbp was constructed. Using a cocktail of 41 BACs in three colors, all chromosome pairs could be individualized simultaneously. Seven ancestral blocks emerged from duplicated chromosome segments of 19 *Spirodela* chromosomes. The chromosomally integrated genome of *S.polyrhiza* and the established prerequisites for comparative chromosome painting enable future studies on the chromosome homoeology and karyotype evolution of duckweed species.

RNA editing in chloroplasts of *Spirodela polyrhiza*, an aquatic monocotelydonous species

Wang, WQ; Zhang, W; Wu, YR; Maliga, P; Messing, J

PLOS ONE 10: Article Number: e0140285; DOI: 10.1371/journal.pone.0140285 (2015)

RNA editing is the post-transcriptional conversion from C to U before translation, providing a unique feature in the regulation of gene expression. Here, we used a robust and efficient method based on RNA-seq from non-ribosomal total RNA to simultaneously measure chloroplast-gene expression and RNA editing efficiency in the Greater Duckweed, *Spirodela polyrhiza*, a species that provides a new reference for the phylogenetic studies of monocoty-ledonous plants. We identified 66 editing sites at the genome-wide level, with an average editing efficiency of 76%. We found that the expression levels of chloroplast genes were relatively constant, but 11 RNA editing sites show significant changes in editing efficiency, when fronds turn into turions. Thus, RNA editing efficiency contributes more to the yield of translatable transcripts than steady state mRNA levels. Comparison of RNA editing sites in coconut, Spirodela, maize, and rice suggests that RNA editing originated from a common ancestor.

Physiology

How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus *Wolffia* (Lemnaceae)

Sree, KS; Sudakaran, S; Appenroth, KJ

ACTA PHYSIOLOGIAE PLANTARUM 37, Article Number: 204, DOI: 10.1007/s11738-015-1951-3 (2015)

Species of the genus *Wolffia* (duckweed) are harvested from natural water bodies in many countries for human consumption. Relative growth rates (RGR) of 25 clones (ecotypes) representing all 11 species of the genus *Wolffia* were investigated under standardized laboratory conditions in search for potential candidates for production of *Wolffia* biomass at a biotechnological scale. This is the first report of large-scale screening of physiological properties of *Wolffia* species. Large differences in RGR of different clones were detected, e.g., in *Wolffia globosa*. Interestingly, intraspecific differences, i.e., at the level of clones are much higher than differences between species. Rate of photosynthesis (oxygen production in light) and respiration (oxygen consumption in dark) in clones of *W. globosa*, measured under standardized conditions, are in positive correlation with their respective RGR. Higher rate of photosynthesis seems to be a determining factor for higher RGR. The RGR of the first available axenic clone of the re-discovered species, *Wolffia microscopica* (clone 2005), depends strongly on the nutrient medium used, in contrast to other investigated species. This clone of *W. microscopica* has a doubling time of 29.3 h and represents the fastest growing flowering plant known till date.

Optimization of conditions (pH and temperature) for *Lemna gibba* production using fuzzy model coupled with Mamdani's method

Suthar, S; Verma, R; Deep, S; Kumar, K

ECOLOGICAL ENGINEERING 83: 452-455 (2015)

A fuzzy-logic-based diagnosis system was developed to determine the effect of pH and temperature on duckweed *Lemna gibba* biomass production. The measured data of variables were implemented into the fuzzy inference system (FIS) with Mamdani's method. A fuzzy rule-based model was shaped to define the essential quality parameters monitored as pH and temperature as inputs. The fuzzy modeled values of biomass gain were validated against the experimental values with a strong correlation r2 of 0.98.

Phytoremediation

Phytoremediation potential of *Lemna minor L.* for heavy metals

Bokhari, SH; Ahmad, I; Mahmood-Ul-Hassan, M; Mohammad, A

INTERNATIONAL JOURNAL of PHYTOREMEDIATION 18: 25-32 (2016)

Phytoremediation potential of *L. minor* for cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) from two different types of effluent in raw form was evaluated in a glass house experiment using hydroponic studies for a period of 31days. Heavy metals concentration in water and plant sample was analyzed at 3, 10, 17, 24, and 31day. Removal efficiency, metal uptake and bio-concentration factor were also calculated. Effluents were initially analyzed for physical, chemical and microbiological parameters and results indicated that municipal effluent (ME) was highly contaminated in terms of nutrient and organic load than sewage mixed industrial effluent (SMIE). Results confirmed the accumulation of heavy metals within plant and subsequent decrease in the effluents. Removal efficiency was greater than 80% for all metals and maximum removal was observed for nickel (99%) from SMIE. Accumulation and uptake of lead in dry biomass was significantly higher than other metals. Bio-concentration factors were less than 1000 and maximum BCFs were found for copper (558) and lead (523.1) indicated that plant is a moderate accumulator of both metals. Overall, *L. minor* showed better performance from SMIE and was more effective in extracting lead than other metals.

Cultivating duckweed *Lemna minor* in urine and treated domestic wastewater for simultaneous biomass production and removal of nutrients and antimicrobials

Iatrou, EI; Stasinakis, AS; Aloupi, M

Duckweed *Lemna minor* was cultivated in human urine (HU) and the effect of urine type, dilution factor, temperature, existence of macro- and microelements on growth rate was investigated. The simultaneous removal of nutrients and selected antimicrobials was also studied in experiments with HU and treated domestic wastewater, while the starch and protein content of biomass was determined. Higher growth rates were observed at 24 degrees C, using HU stored for 1 d and with dilution factor equal to 1:200. In experiments with HU and wastewater, the removal of COD, total phosphorus and total nitrogen exceeded 80%, 90% and 50%, respectively, while ciprofloxacin and sulfamethoxazole were eliminated by more than 80%. The main removal mechanism for the former antimicrobial was photodegradation, while plant uptake and biodegradation seem to be of significant importance for the latter. Crude protein content reached 31.6% in experiments with HU and biomass harvesting, while starch content was enhanced when duckweed was transferred to water for 21 d, reaching 47.1%.

The potential of *Lemna gibba L.* and *Lemna minor L.* to remove Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey

Sasmaz, M; Topal, EIA; Obek, E; Sasmaz, A

JOURNAL of ENVIRONMENTAL MANAGEMENT 163: 246-253 (2015)

This study was designed to investigate removal efficiencies of Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey by *Lemna gibba L.* and *Lemna minor L.* These plants were placed in the gallery water of Keban Pb Zn ore deposits and adapted individually fed to the reactors. During the study period (8 days), the plant and water samples were collected daily and the temperature, pH, and electric conductivity of the gallery water were measured daily. The plants were washed, dried, and burned at 300 degrees C for 24 h in a drying oven. These ash and water samples were analyzed by ICP-MS to determine the amounts of Cu, Pb, Zn, and As. The Cu, Pb, Zn and As concentrations in the gallery water of the study area detected 67, 7.5, 7230, and 96 mu g L-1, respectively. According to the results, the obtained efficiencies in *L. minor L.* and *L. gibba L.* are: 87% at day 2 and 36% at day 3 for Cu; 1259% at day 2 and 1015% at day 2 for Pb; 628% at day 3 and 382% at day 3 for Zn; and 7070% at day 3 and 19,709% at day 2 for As, respectively. The present study revealed that both *L. minor L.* and *L. gibba L.* had very high potential to remove Cu, Pb, Zn, and As in gallery water contaminated by different ores.

Carbon and energy fixation of great duckweed *Spirodela polyrhiza* growing in swine wastewater

Wang, WG; Yang, C; Tang, XY; Zhu, QL; Pan, K; Cai, DG; Hu, QC; Ma, DW

ENVIRONMENTAL SCIENCE and POLLUTION RESEARCH 22: 15804-15811 (2015)

The ability to fix carbon and energy in swine waste-water of duckweeds was investigated using *Spirodela polyrhiza* as the model species. Cultures of *S. polyrhiza* were grown in dilutions of both original swine wastewater (OSW) and anaerobic digestion effluent (ADE) based on total ammonia nitrogen (TAN). Results showed that elevated concentrations of TAN caused decreased growth, carbon fixation, and energy production rates, particularly just after the first rise in two types of swine wastewater. Also, OSW was more suitable for *S. polyrhiza* cultivation than ADE. Maximum carbon and energy fixation were achieved at OSW-TAN concentrations of 12.08 and 13.07 mg L-1, respectively. Photosynthetic activity of *S. polyrhiza* could be inhibited by both nutrient stress (in high-concentration wastewater) and nutrient limitation (in low-concentration wastewater), affecting its growth and ability for carbon-energy fixation.

Performance assessment of aquatic macrophytes for treatment of municipal wastewater

Shah, M; Hashmi, HN; Ghumman, AR; Zeeshan, M

JOURNAL of the SOUTH AFRICAN INSTITUTION of CIVIL ENGINEERING 57: 18-25 (2015)

The objective of the study was to evaluate the performance of three different aquatic macrophytes for treatment of municipal wastewater collected from Taxila in Pakistan. A physical model of a typical treatment plant was constructed and was operated for six experimental runs with each species of macrophyte. Every experimental run consisted of a thirty-day period. Regular monitoring of influent and effluent concentrations were made during each experimental run. Locally available macrophyte species (water hyacinth, duckweed and water lettuce) were selected for testing. To evaluate the treatment performance of each macrophyte, BOD5, COD, and nutrients (nitrogen and phosphorus) were monitored in the effluent from the model at different detention times for each experimental run, after having ensured steady state conditions. The average reduction of effluent value for each parameter, using water hyacinth, was 50.61% for BOD5, 46.38% for COD, 40.34% for nitrogen and 18.76% for phosphorus. For duckweed, the average removal efficiency for the selected parameters was 33.43% for BOD5, 26.37% for COD, 17.59% for nitrogen and 15.25% for phosphorus, and for water lettuce the average removal efficiency was 33.43% for BOD5, 26.37% for COD, 17.59% for nitrogen and 15.25% for phosphorus. The mechanisms of pollutant removal in this system include both aerobic and anaerobic microbiological conversions, sorption, sedimentation, volatilisation and chemical transformations. The rapid growth of the biomass was measured within the first ten days of the detention time. It was also observed that performance of macrophytes is influenced by variations in pH and temperature. A pH of 6-9 and temperature of 15-38 degrees C is most favourable for treatment of wastewater by macrophytes.

Biosorption and biomineralization of uranium(VI) from aqueous solutions by *Landoltia punctata*

Nie, XQ; Dong, FQ; Liu, N; Zhang, D; Liu, MX; Yang, J; Zhang, W

SPECTROSCOPY and SPECTRAL ANALYSIS 35: 2613-2619 (2015)

The biosorption and biomineralization characteristics of uranium by the duckweed *Landoltia punctata* was investigated in aqueous solutions enriched with 1 to 250 mg . L-1 of U(VI) supplied as uranyl nitrate [UO2(NO3)(2) center dot 6H(2)O]. The maximum uranium removal for the plant cultivar occurred at pH 4 similar to 5 of solution and their uranium removal efficiencies exceeded 90% after 24 h. In kinetics studies, the dried powder of duckweed can finished nearly 80% adsorption within 5 min, the batch adsorption equilibrium can be reached within 24 h for the living and dried powder of duckweed, Both for the living and dried powder of duckweed, the experimental data were well fitted by the pseudo-second-order rate model with the degree of fitting (r) higher than 0.99. The adsorption isotherms could be better described by the Freundlich model than the Langmuir model. In addition,

Fourier transform infrared spectroscopy (FTIR) revealed that the surface of *Landoltia punctata* possess many active groups such as hydroxyl, carboxyl, phosphate and amide groups, the hydroxyl, amino groups involved in adsorption of U(VI) by living and dried powder of *Landoltia punctata*, and the phosphate groups also participated in the adsorption behavior of U(VI) by the living *Landoltia punctata*. The living *Landoltia punctata* reduction part of U(VI) to U(IV) was observed by XPS analysis. SEM and energy dispersive X-ray spectroscopy (EDS) of duckweed from 10 similar to 200 mg . L-1 uranium treatments indeed showed root surface of living *Landoltia punctata* formed a significant portion of U precipitates with nanometer sized schistose structures that consisted primarily U and P, not containing C. Inorganic phosphate was released by the root cells of *Landoltia punctata* during the experiments providing ligands for formation of insoluble U(VI) and U(IV) phosphates. The distinct uranium peaks in the EDS spectra of the cluster on the root surface can be observed after biosorption and the uranium and phosphorus mass ratio of the cluster spot was measured to be 82.5% and 8.76% of the total component weight, respectively, and the atomic percentage of 30.89% and 25.19%, respectively. It is worth noting that the phosphorus mass ratio and the atomic rate of the control group is only 0.24% and 0.11%, respectively. But there was no similar crystals observed on the surface of dried powder of *Landoltia punctata* after biosorption. The present work suggests that living and dried powder of *Landoltia punctata* can remove more than 90% U(VI) from solution simultaneously precipitated together with phosphate by the living *Landoltia punctata*, and the dried powder of *Landoltia punctata* adsorption U(VI) is mainly through the effect of electrostatic attraction, ion exchange and complexation coordination, etc. Here, for the first time, the presence of U immobilization mechanisms within one aquatic plant is reported using *Landoltia punctata*.

Phytotoxicity

Possible ecological risk of two pharmaceuticals diclofenac and paracetamol demonstrated on a model plant *Lemna minor*

Kummerova, M; Zezulka, S; Babula, P; Triska, J

Journal of Hazardous Materials 302:351-61 (2016)

Lemna minor is often used in environmental risk assessment and it can be supposed that usually evaluated parameters will be reliable even for assessing the risk of pharmaceuticals. Subtle changes in duckweed plant number, biomass production, and leaf area size induced by 10-day-exposure to diclofenac (DCF) and paracetamol (PCT) (0.1, 10, and 100mug/L), excepting 100mug/L DCF, are in contrast with considerable changes on biochemical and histochemical level. Both drugs caused a decrease in content of photosynthetic pigments (by up to 50%), an increase in non-photochemical quenching (by 65%) and decrease in relative chlorophyll fluorescence decay values (by up to 90% with DCF). Both DCF and especially PCT increased amount of reactive nitrogen and oxygen species in roots. DCF-induced effects included mainly increased lipid peroxidation (by 78%), disturbation in membrane integrity and lowering both oxidoreductase and dehydrogenase activities (by 30%). PCT increased the content of soluble proteins and phenolics. Higher concentrations of both DCF and PCT increased the levels of oxidised ascorbate (by 30%) and oxidised thiols (by up to 84% with DCF). Glutathion-reductase activity was elevated by both pharmaceuticals (nearly by 90%), glutathion-S-transferase activity increased mainly with PCT (by 22%). The early and sensitive indicators of DCF and PCT phytotoxicity stress in duckweed are mainly the changes in biochemical processes, connected with activation of defense mechanisms against oxidative stress.

Ibuprofen exposure in *Lemna gibba L.*: Evaluation of growth and phytotoxic indicators, detection of ibuprofen and identification of its metabolites in plant and in the medium

Pietrini, F; Di Baccio, D; Acena, J; Perez, S; Barcelo, D; Zacchini, M

Journal of Hazardous Materials 300:189-93 (2015)

Ibuprofen (IBU) is detected worldwide in water bodies due to the incomplete removal by wastewater treatments. Contrasting results have been reported on the toxicity of IBU on aquatic biomonitor plants such as duckweed, and no data about IBU detection and metabolism in plants has been reported. In this work, the effects of 1mgL(-1) IBU on *Lemna gibba L.* were monitored in an 8-day laboratory test. In particular, an increase in frond number (+12%) and multiplication rate (+10%) while no variations in photosynthetic pigment content were observed. Moreover, UPLC-HRMS analysis of the presence of IBU and its metabolites in plants and in the growth medium was performed. The results showed that, besides IBU, 11 IBU metabolites were detected in plants. Among the IBU metabolites, hydroxyl- and dihydroxyl-IBU were found, whereas carboxyl-IBU was undetectable. Interestingly, some IBU metabolites were detected in the plant growth solution at the end of the IBU treatment, while no IBU products were found in the IBU solution without plants, suggesting a role for *L. gibba* in IBU metabolism. The findings of this work represent an important step for a better evaluation of the effects of IBU and its metabolites in duckweed, with notable implications for the eco-toxicological assessment of IBU in the aquatic ecosystem.

Utility of Duckweeds as Source of Biomass Energy: a Review

Verma, R; Suthar, S

BIOENERGY RESEARCH 8: 1589-1597 (2015)

The quest for alternative sources of energy has evoked the interest in exploring potentials of living biological wastes as new energy materials. Duckweeds are produced abundantly as weeds in freshwater surface bodies and can be a source of biomass for bioenergy productions. There are approximately 40 species of this group worldwide belonging to five genera (*Spirodela*, *Lemna*, *Wolfiella*, *Wolffia* and *Landoltia*). The structural peculiarities (small plant size, limited life cycle, high duplication rate, etc.) and chemical characteristics (dry weight basis): 17.6-35 % (carbohydrate), 21-38 % (starch), 16-41.7 % (crude protein), 8.8-15.6 % (crude fibre) and 4.5-9 % (lipid) make duckweed as possible feedstock for biomass-based energy operations. The high contents of valuable fatty acids (palmitic acid and linoleic acid) and starch (3-75 %) in duckweed biomass suggest its utility in biorefinery. Recent lab-scale studies have shown remarkable results in terms of energy yield during the processes like anaerobic digestion, incineration, pyrolysis, gasification, oxidation, etc. Another good quality of duckweeds is its hyperaccumulative properties for a variety of water pollutants. Therefore, this group of weeds has been recommended widely for designing on-site phytoremediation system for community wastewater treatment. Thus, duckweed technology can be adopted as coupled technology to harness two environmental approaches, i.e. wastewater treatment and energy biomass production for sustainable development of the human society.

The enzymatic and antioxidative stress response of *Lemna minor* to copper and a chloroacetamide herbicide

Obermeier, M; Schroder, CA; Helmreich, B; Schroder, P

Environmental Science and Pollution Research International 22:18495-507 (2015)

Lemna minor L., a widely used model plant for toxicity tests has raised interest for its application to phytoremediation due to its rapid growth and ubiquitous occurrence. In rural areas, the pollution of water bodies with heavy metals and agrochemicals poses a problem to surface water quality. Among problematic compounds, heavy metals (copper) and pesticides are frequently found in water bodies. To establish duckweed as a potential plant for phytoremediation, enzymatic and antioxidative stress responses of *Lemna minor* during exposure to copper and a chloroacetamide herbicide were investigated in laboratory studies. The present study aimed at evaluating growth and the antioxidative and glutathione-dependent enzyme activity of *Lemna* plants and its performance in a scenario for phytoremediation of copper and a chloroacetamide herbicide. *Lemna minor* was grown in Steinberg medium under controlled conditions. Plants were treated with CuSO4 (ion conc. 50 and 100mug/L) and pethoxamide (1.25 and 2.5mug/L). Measurements following published methods focused on plant growth, oxidative stress, and basic detoxification enzymes. Duckweed proved to survive treatment with the respective concentrations of both pollutants very well. Its growth was inhibited scarcely, and no visible symptoms occurred. On the cellular basis, accumulation of O2 (-) and H2O2 were detected, as well as stress reactions of antioxidative enzymes. Duckweed detoxification potential for organic pollutants was high and increased significantly with incubation. Pethoxamide was found to be conjugated with glutathione. Copper was accumulated in the fronds at high levels, and transient oxidative defense reactions were triggered. This work confirms the significance of *L. minor* for the removal of copper from water and the conjugation of the selective herbicide pethoxamide. Both organic and inorganic xenobiotics induced different trends of enzymatic and antioxidative stress response. The strong increase of stress responses following copper exposure is well known as oxidative burst, which is probably different from the much more long-lasting responses found in plants exposed to pethoxamide. *Lemna sp.* might be used as a tool for phytoremediation of low-level contamination with metals and organic xenobiotics, however the authors recommend a more detailed analysis of the development of the oxidative burst following copper exposure and of the enzymatic metabolism of pethoxamide in order to elucidate the extent of its removal from water.

Antioxidative stress responses in the floating macrophyte *Lemna minor L.* with cylindrospermopsin exposure

Flores-Rojas, NC; Esterhuizen-Londt, M; Pflugmacher, S

Aquatic Toxicology 169:188-95 (2015)

Cylindrospermopsin toxicity and oxidative stress have been examined in aquatic animals, however, only a few studies with aquatic plants have been conducted focusing on the potential for bioaccumulation of cylindrospermopsin. The oxidative stress effects caused by cylindrospermopsin on macrophytes have not yet been specifically studied. The oxidative stress response of *Lemna minor L.* with exposure to cylindrospermopsin, was therefore tested in this study. The hydrogen peroxide concentration together with the activities of the antioxidant enzymes (catalase, peroxidase, glutathione reductase and glutathione S-transferase) were determined after 24h (hours) of exposure to varying concentrations (0.025, 0.25, 2.5 and 25mug/L) of cylindrospermopsin. Responses with longer exposure periods (48, 96, 168h) were tested only with exposure to 2.5 and 25mug/L cylindrospermopsin. Additionally, the content of the carotenoids was determined as a possible non-enzymatic antioxidant defence mechanism against cylindrospermopsin. The levels of hydrogen peroxide increased after 24h even at the lowest cylindrospermopsin exposure concentrations. Catalase showed the most representative antioxidant response observed after 24h and maintained its activity throughout the experiment. Catalase activity corresponded with the contents of hydrogen peroxide at 2.5 and 25mug/L cylindrospermopsin. The data suggest that glutathione S-transferase, glutathione reductase and the carotenoid content act together with catalase but are more sensitive to higher concentrations of cylindrospermopsin and after a longer exposure period (168h). The results indicate that cylindrospermopsin promotes oxidative stress in *L. minor* at concentrations of 2.5 and

25mug/L. However, *L. minor* has sufficient defence mechanisms in place against this cyanobacterial toxin. Even though L. minor exhibits the potential to managing and control cylindrospermopsin contamination in aquatic systems, further studies in tolerance limits to cylindrospermopsin, uptake and experiments with prolonged exposure periods of more than 7 days are required.

The effect of sulfate on selenate bioaccumulation in two freshwater primary producers: A duckweed (*Lemna minor*) and a green alga (*Pseudokirchneriella subcapitata*)

Lo, BP; Elphick, JR; Bailey, HC; Baker, JA; Kennedy, CJ

Environmental Toxicology and Chemistry / SETAC 34:2841-5 (2015)

Predicting selenium bioaccumulation is complicated because site-specific conditions, including the ionic composition of water, affect the bioconcentration of inorganic selenium into the food web. Selenium tissue concentrations were measured in *Lemna minor* and *Pseudokirchneriella subcapitata* following exposure to selenate and sulfate. Selenium accumulation differed between species, and sulfate reduced selenium uptake in both species, indicating that ionic constituents, in particular sulfate, are important in modifying selenium uptake by primary producers.

Characterizing dose response relationships: Chronic gamma radiation in *Lemna minor* induces oxidative stress and altered polyploidy level

Van Hoeck, A; Horemans, N; Van Hees, M; Nauts, R; Knapen, D; Vandenhove, H; Blust, R

JOURNAL of ENVIRONMENTAL RADIOACTIVITY 150: 195-202 (2015)

The biological effects and interactions of different radiation types in plants are still far from understood. Among different radiation types, external gamma radiation treatments have been mostly studied to assess the biological impact of radiation toxicity in organisms. Upon exposure of plants to gamma radiation, ionisation events can cause, either directly or indirectly, severe biological damage to DNA and other biomolecules. However, the biological responses and oxidative stress related mechanisms under chronic radiation conditions are poorly understood in plant systems. In the following study, it was questioned if the *Lemna minor* growth inhibition test is a suitable approach to also assess the radiotoxicity of this freshwater plant. Therefore, *L. minor* plants were continuously exposed for seven days to 12 different dose rate levels covering almost six orders of magnitude starting from 80 mu Gy h(-1) up to 1.5 Gy h(-1). Subsequently, growth, antioxidative defence system and genomic responses of *L. minor* plants were evaluated. Although *L. minor* plants could survive the exposure treatment at environmental relevant exposure conditions, higher dose rate levels induced dose dependent growth inhibitions starting from approximately 27 mGy h(-1). A ten-percentage growth inhibition of frond area Effective Dose Rate (EDR10)) was estimated at 95 +/- 7 mGy h(-1), followed by 153 +/- 13 mGy h(-1) and 169 +/- 12 mGy h(-1) on fresh weight and frond number, respectively. Up to a dose rate of approximately 5 mGy h-1, antioxidative enzymes and metabolites remained unaffected in plants. A significant change in catalase enzyme activity was found at 27 mGy h(-1) which was accompanied with significant increases of other antioxidative enzyme activities and shifts in ascorbate and glutathione content at higher dose rate levels, indicating an increase in oxidative stress in plants. Recent plant research hypothesized that environmental genotoxic stress conditions can induce endoreduplication events. Here an increase in ploidy level was observed at the highest tested dose rate. In conclusion, the results revealed that in plants several mechanisms and pathways interplay to cope with radiation induced stress.

Links for Further Reading

www.Lemnapedia.org Online developing compendium of duckweed research & applications, founded by the ISCDRA.

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

www.InternationalLemnaAssociation.org Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://www.duckweed.ch The Landolt Duckweed Collection

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants

Note to the Reader

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