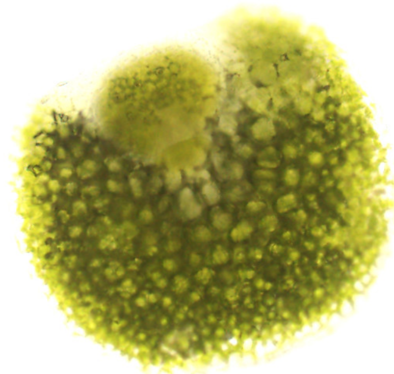
Newsletter of the Community of Duckweed Research and Applications, edited by the ISCDRA

DUCKWEED FORUM

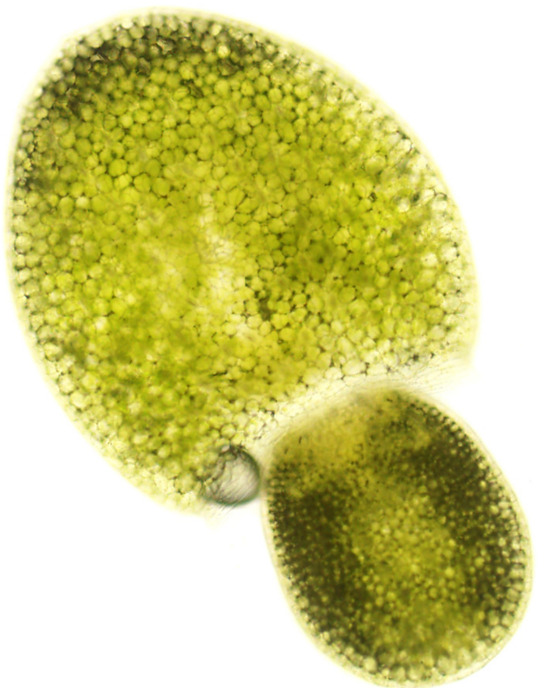
ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 4 (3), issue 14, pages 272 - 303

Wolffia globosa 9297



Wolffia microscopica 2005



Wolffia brasiliensis 8116

Wolffia australiana 8730

1 mm

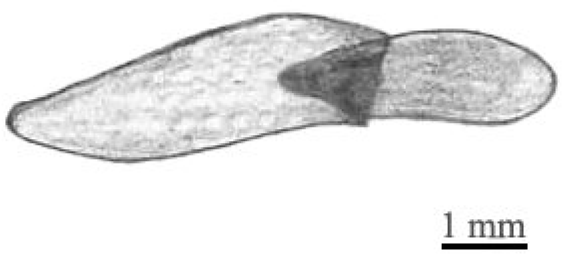
These four duckweed species belong to the genus *Wolffia*, with a high reduction in their morphology. *Wolffia globosa* is a common and sometimes even a dominant species in South and Southeast Asia and is also found in South Africa. Whereas the re-discovered species *W. microscopica* is endemic to India, Bangladesh and Pakistan. *Wolffia australiana*, having the smallest genome in this genus, could be found in Australia and New Zealand, while *W. brasiliensis* appears to be a species dispersed throughout the warmer regions of the American continents.
Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University).

In this issue

International Steering Committee on Duckweed Research and Applications Members

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

Science meets art: *Wolffiella oblonga*

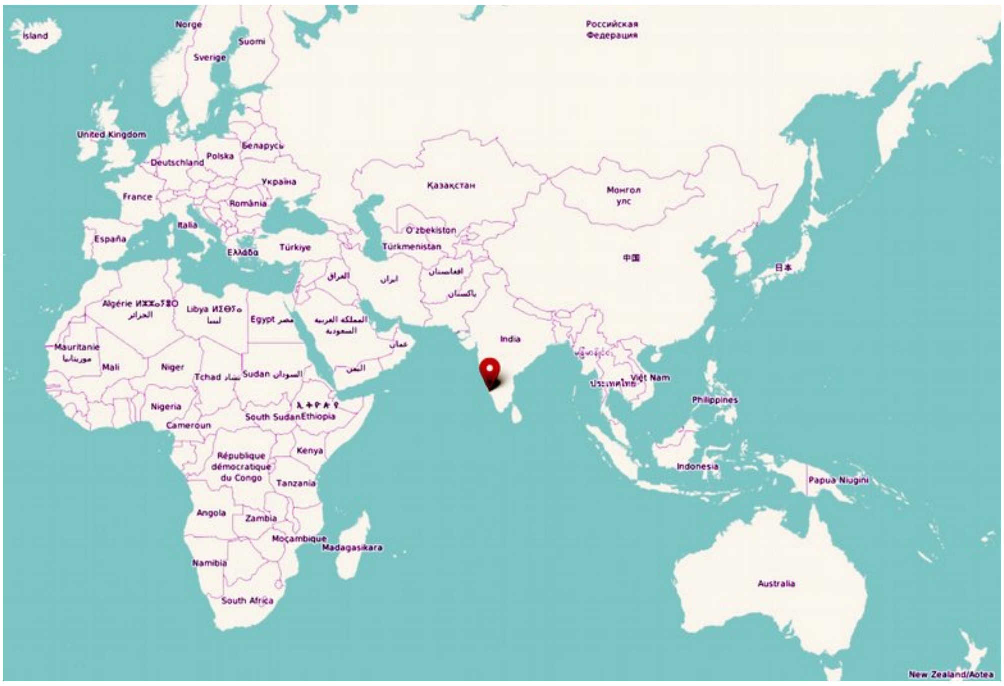


Wolffiella oblonga (Phil.) Hegelm., native to the warm regions of South and North America, impresses by its elegant appearance: narrow tongue-shaped with a rounded tip. It occasionally forms colonies that are often star-like although not as frequently as the closely related *W. gladiata* [Chin J Appl. Environ Biol 19: 1-10 (2013)], a drawing of which was used until recently as the icon of the ISCDRA. Drawing by Dr. K. Sowjanya Sree, Dept. of Environmental Science, Central University of Kerala, Padannakad, India

Letter from the editor

Dear friends of duckweed,

This issue of our Newsletter "Duckweed Forum" invites you to attend the 4th International Conference on Duckweed Research and Applications from 23rd – 26th October, 2017 in Periye, Kerala, India. The conference will be organized by the chair Dr. K. Sowjanya Sree from the Central University of Kerala and the co-Chair, Prof. Dr. Jitendra P. Khurana from the University of Delhi South Campus. We present to you the first circular. When you have further questions, please contact the chair of the conference at the given address. We welcome you all next year to Kerala, India.



You will find in this issue a report of an excursion to Thailand, a country where duckweeds have been a part of the human diet for many generations.

In the "Discussion Corner", we continue our joint effort to standardize common methods in duckweed research, such as taxonomic terminology, measuring growth rate, phytotoxicity test, turion formation and starch determination. We are looking forward to have further suggestions from our community. In the section "Student Spotlight", Ms. Philomena Chu from the Rutgers University in New Jersey explains her scientific interest and her work with duckweed. Further in the "Useful Method" section, two representatives from the LemnaTec Company in Aachen, Germany, explain how phenotypic and phytotoxicity investigations can be improved using automated image analysis. As usual, we give an overview of the scientific duckweed literature published in the past 4 months in the last section of "From the Database".

You might have already noticed that the front page presents again four photos of different duckweed species. Under the "Science meets Art" section in the page with the Table of Contents, the species *Wolffiella oblonga* is also introduced.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),

Klaus-J. Appenroth, Chair

4th International Conference on Duckweed Research and Applications (ICDRA-2017)

Tentative dates: 23-26 October, 2017

Department of Environmental Science, Central University of Kerala, India

1st Announcement

Patron
Prof. Dr. G. Gopa Kumar
Vice Chancellor, Central University of Kerala

International Organizing Committee
Dr. Klaus-J. Appenroth, Head of ISCDRA
FS University of Jena, Germany
Prof. Dr. Eric Lam, Rutgers University, USA
Prof. Dr. Marvin Edelman,
Weizmann Institute of Science, Israel
Dr. Autar Mattoo, USDA-ARS, USA
Prof. Dr. Jiaming Zhang, CATAS, China
Dr. Tokitaka Oyama, Kyoto University, Japan
Ms. Tamra Fakhoorian, ILA, USA
...more to join

Local organizing committee
Dr. M. Muthukumar, Dean, school of Earth Science Systems, CUK
Dr. J. Sangeetha, Dept. of Environmental Science, CUK
...more to join

Chair of the conference
Dr. K. Sowjanya Sree
Assistant Professor
Department of Environmental Science
Central University of Kerala
Padannakad, India

Co-Chair of the conference
Prof. Dr. Jitendra P. Khurana
Department of Plant Molecular Biology
University of Delhi South Campus
New Delhi, India

About the conference:
Duckweeds, small and fast growing aquatic plants belonging to Lemnaceae, are gaining attention both from basic research and application perspective. The first three conferences held biennially starting from 2011 in China, USA and Japan respectively have witnessed a growing interest of the scientific and industrial community in advancing the basic research of duckweeds together with realizing its numerous applications. We hope that this conference would provide a boost for the emerging duckweed research and also wish that it would foster the interactions between academia and industry.

Venue: Central University of Kerala is located in Periye, Kasaragod (District) which is the northern most district of Kerala. Kerala is the southern most state of India and is popularly known as the God's own country for the natural beauty embedded in it. The University campus itself is located in a serene environment with the Western Ghats (one of the three biodiversity hotspots in the country) running parallel to the west coastline of India.

Accommodation:
Good budget hotels are located in Kanhangad and Kasaragod. Apart from hotels, high-end sea-side resorts are also located at a convenient distance from the University campus.

Registrations and Abstract submissions will open soon

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It is our pleasure to dedicate the 4th ICDRA (2017) to Prof. Satish C. Maheshwari, University of Rajasthan, Jaipur, India

Duckweed science and food excursion in Thailand

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Since the 1971 report of Krachang Bhanthumnavin and Michael G. McGarry from Bangkok, Thailand in 'Nature', it became widely known that duckweed is used as human food in some of the Southeast Asian countries like Thailand, Laos and Cambodia. With the growing interest to use duckweed and duckweed-based protein as human food, we were very enthusiastic to accept the invitation of Dr. Metha Meetam from the Department of Biology, Mahidol University, Bangkok, Thailand, to catch a glimpse of the duckweed being grown, harvested and sold in the food markets. We started on 25th of March, 2016 with a workshop on "Duckweed research and applications".

Ms. Orathai Pakdee

Approximately 50 participants from all over Thailand attended this meeting. Dr. Klaus-J. Appenroth and Dr. K. Sowjanya Sree presented the state of the art of duckweed research, especially following the 3rd International Conference on Duckweed Research and Applications in Kyoto, Japan in 2015. Thereafter, K. Sowjanya Sree gave an introduction into the key for determination of duckweed genera and species with practical demonstration. Although duckweed was not available in the markets in Bangkok, especially in March or April, it could be ordered from the north of Thailand where it is more commonly eaten. We bought 5kg of Wolffia (shown in picture) from the market as pre-ordered by Metha Meetam. We were informed that the market price of Wolffia is 10-15 Thai baht (25-40 cents according to the current exchange rate to Euro) per kilogram when it is in season and is 50-80 Thai baht (1.3-2.0 Euro) during off-season.

While Bhanthumnavin and McGarry originally reported that the duckweed being eaten in Thailand is *Wolffia arrhiza*, we agree with the suspicion of the late Prof. Elias Landolt (1986) that it is *W. globosa*, which is commonly occurring in this region. One of the students, Ms. Orathai Pakdee, who hails from the northern part of Thailand with a tradition of eating duckweed, made delicious Wolffia snacks for the high tea during our workshop. One was an omelet with Wolffia that was served with a sauce (left plate in picture); and the other a thick soup of Wolffia garnished with onion, tomato, green chili and fresh coriander leaves (right plate in picture).

It could be used as a topping on a wafer, crisp or a biscuit. In the Wolffia-and-egg omelet, the little water eggs (as they are called in Thai language) made the omelet more appealing to the eyes as well as to the pallet. In the soup, even after boiling, the granular nature of Wolffia still remained and being a major ingredient, the presence of Wolffia could be felt predominantly. In both cases, of course, the dish took the taste from the rest of the ingredients since Wolffia itself is rather mild. We immensely enjoyed these dishes as did the native Thai participants.

Photo A: From left, Dr. Metha Meetam, Ms. Chuenduangjai Khongban, Dr. K. Sowjanya Sree, Dr. Klaus-J. Appenroth

The next day we started on a two-day excursion to the northern part of Thailand where Wolffia is harvested and consumed commonly by the local people. First we visited the Office of Agricultural Extension and Development Region 7 (Nakhon Ratchasima), an institution that tries to propagate the use of duckweed as a healthy food and to educate the local farmers on duckweed cultivation. Ms. Chuenduangjai Khongban explained in detail about their method of cultivation of Wolffia. Later, guided by Ms. Khongban, we visited some of the ponds to see how Wolffia is grown in this region. We also stopped by to look at the natural population of duckweeds in this region and could find *Spirodela polyrhiza*, *Landoltia punctata* and *Lemna aequinoctialis* aside from *W. globosa*. Although the name "kai-nam" (which means eggs of water) became more famous in the world outside Thailand, kai-pum and kai-nhae are its synonyms which are used in different regions of Thailand. We would like to thank Dr. Metha Meetam, who made this unique experience possible and to all his students (shown in the last photo) who assisted us.

Discussion corner: Standardization of protocols for duckweed research

Klaus-J. Appenroth[1], Eric Lam[2]

[1]Institute of General Botany and Plant Physiology, University of Jena, Germany

[2]Plant Biology & Pathology, Rutgers, the State University of New Jersey, New Brunswick, USA

To proceed further with the standardization of protocols for duckweed research and applications in our community, we discuss the following items.

Taxonomic terminology

Most of us agreed that we should consider duckweed as a family using the term "Lemnaceae" instead of considering them as a subfamily of Araceae using the term "Lemnoideae". Presently, 37 species were described within five monophyletic genera. The correct species names are summarized in one of the papers given below. The paper of Sree, Bog and Appenroth (2016) provides a list of many invalid species names for duckweed strains reported in the literature and their corresponding correct names.

All manuscripts using new axenic ("sterile") clones of duckweed should be validated in their species designation via morphological or molecular markers and registered to obtain a 4 digit code number before publishing so that other researchers could repeat the experiments with the same clone. This would facilitate future comparison of data reported in the literature related to duckweed and will minimize the proliferation of many different coding systems and nomenclature used by researchers in the field.

Registration is now easily done via the **Rutgers Duckweed Stock Cooperative** in New Jersey (managed by the group of Prof. Eric Lam, see http://www.ruduckweed.org).

References:

- Appenroth KJ, Borisjuk N, Lam E (2013) *Telling duckweed apart: Genotyping technologies for Lemnaceae*. Chinese Journal of Applied and Environmental Biology 19: 1-10
- Appenroth KJ, Crawford DJ, Les DH (2015) *After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm?* Plant Biology 17(Suppl 1): 1-4
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Duckweed growth rate determination

When duckweed growth rates are presented in a scientific way, then the growth conditions should ideally be comparable between different studies by various researchers. This would allow the field to leverage the data resources produced by the community as a whole and minimize redundant work. When there are no specific reasons for using a particular set of growth parameters, we suggest the following conditions should be used if at all possible:

25°C, continuous light (24 hour [h] per day), light intensity 100 µmol photons m^{-2} s^{-1}, well-defined inorganic nutrient medium such as the commercially available Schenk & Hildebrandt (SH) salts. Several useful nutrient media were described in our newsletter Duckweed Forum 3: 180-186 (2015) as well.

When photoperiods shorter than 24 h per day were used, the periodic response of the measured parameter (e.g. starch content) must be tested. Only when there are no periodic changes light/darkness, parameters can be measures as in continuous light. "Lux" is not suitable as dimension of light intensity as this unit is adapted from human vision, not to responses to plants. In plants the normal form of measurements should by "Photosynthetic Active Radiation" (PAR) which has to be given as µmol photons m^{-2} s^{-1}. Only in very special experiments, radiation energy can be given as W m^{-2} together with the emission spectrum of the light source.

Duckweed growth must be given as exponential "relative growth rates" (RGR or just GR) with the dimension d^{-1} or h^{-1}. Data as percentage growth supposes a linear growth of duckweed, which is in reality exponential. To make the results easier to understand, doubling time or specific yield (Ziegler et al., 2015) can be calculated from the same data set.

References

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- Sree, KS, Sudakaran S, Appenroth K-J. 2015. *How fast can angiosperm grow? Species and clonal diversity of growth rates in the genus Wolffia (Lemnaceae).* - Acta Physiologiae Plantarum 37(204): DOI: 10.1007/s11738-015-1951-3.
- Ziegler P, Adelmann K, Zimmer S, Schmidt C, Appenroth K-J. 2015. *Relative in vitro growth rates of duckweeds (Lemnaceae) – the most rapidly growing higher plants*. Plant Biology 17 (Suppl. 1): 33-41.

Phytotoxicity

Standardized phytotoxicity tests with duckweed are currently available. We do not recommend that researchers arbitrarily apply their own methods since their results will be difficult to compare to data from other groups. Beside optimal growth conditions concerning temperature and light (again no photoperiod!), the nutrient medium used is of special importance.

Chelating agents like EDTA or tartrate form also complexes with heavy metals preventing their uptake. As a consequence, apparent toxicity in the presence of chelators could be much lower than it is in their absence.

For heavy metals, the Steinberg medium was optimized to the lowest possible EDTA concentrations. There is an alternative, APHA, which is without any chelator. However, then there will likely be Fe stress since iron uptake requires a chelator. Therefore, we do not recommend the APHA protocol. For each concentration of the heavy metal (or any other stressor), RGR should be determined and thereafter used to create dose (concentration) – response curves for growth inhibition. These graphs can be created on the basis of frond number, fresh weight, and dry weight or frond area, and then used to calculate half-maximal effective concentrations or similar values with standard errors (EC_{50} ± SE, EC_{20} ± SE, EC_{10} ± SE).

These standardized parameters will make quantitative comparisons between different datasets possible.

References:

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- Sree, KS, Keresztes Á, Mueller-Roeber B, Brandt R, Eberius M, Fischer W, Appenroth K-J. 2015. *Phytotoxicity of cobalt ions on the duckweed Lemna minor – Morphology, ion uptake, and starch accumulation*. Chemosphere, 131, 149-156.

Turion formation

Turion formation in *Spirodela polyrhiza* is possible in light and in darkness. In order to compare different clones or a particular clone under different conditions, the ratio between formed turions and the number of fronds has to be calculated at the time when no new turions are formed in the cultivation flask. Turion formation is becoming more important when the new, suggested test methods for phytotoxicity (see "Highlights" in this issue of Duckweed Forum) are adopted on a broad basis. Light intensity, nutrient composition, temperature and medium volume are important factors. Phosphate is needed to support the inoculum for vegetative growth and during active growth phosphate from the medium is rapidly taken up. The resulting lack of external phosphate due to this uptake is sensed by the plant as a signal to start the developmental process of turion formation when a threshold of external phosphate concentration is reached. Turions can be most easily formed when the nutrient medium contains a low concentration of phosphate, e.g. 60 µM or less. Other signals (low sulphate concentrations, low temperature, and addition of the natural phytohormone abscisic acid) also can be a switch for turion formation. Only when all conditions are strictly standardized and the plants are carefully pre-cultivated under the same cultivation conditions, reproducible quantitative results can be obtained and the results can be compared with those of other research groups.

References:

- Appenroth K-J. 2002. *Co-action of temperature and phosphate in inducing turion formation in Spirodela polyrhiza* (Great duckweed). Plant, Cell and Environment 25: 1079–1085.
- Appenroth K-J, Adamec L. 2015. *Specific turion yields of different clones of Spirodela polyrhiza depend on external phosphate thresholds*. Plant Biology 17 (Suppl. 1): 125-129.
- Appenroth K-J, Nickel G. 2010. *Induction of turion formation in Spirodela polyrhiza under close-to-nature conditions: the environmental signals that induce the developmental process in nature*. Physiologia Plantarum 138: 312–320.
- Appenroth K-J, Teller S, Horn M. 1996. *Photophysiology of turion formation and germination in Spirodela polyrhiza*. Biologia Plantarum 38: 95–106.
- Kuehdorf K, Jetschke G, Ballani L, Appenroth K-J. 2013. *The clonal dependence of turion formation in the duckweed Spirodela polyrhiza – an ecogeographical approach*. Physiologia Plantarum 150: 46–54.

Starch content determination

There are several methods for starch determination in use in the duckweed community. We used originally the method of Lustinec et al. (1983) on the basis of precipitation of the starch-iodine complex (Ley et al., 1997). As we had problems with reproducibility, we turned to another method described by Mager et al. (1991) for commercial starch samples and used it for duckweed (Appenroth et al. 2003) in all subsequent papers. A third method bases on the work of Sluiter and Sluiter (2005) has also been used for duckweed analysis e.g. by Zhao et al. (2015). Until now, a systematic comparison of these methods for their accuracy and reproducibility has not been published. Doing this would be a service to the duckweed community for standardizing an important method that can be used routinely to track this key metabolite in Lemnaceae.

References:

- Appenroth K-J, Keresztes A, Sarvari E, Jaglarz A, Fischer W. 2003. *Multiple effects of chromate on Spirodela polyrhiza: Electron microscopy and biochemical investigations*. Plant Biology 5: 315-323.
- Lustinec J, Hadadova V, Kaminek M, Prochazka Z. 1983. *Quantitative determination of starch, amylose, and amylopectin in plant tissues using glass fiber paper*. Analytical Biochemistry 132: 265–271.
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- Zhao Z, Shi HJ, Wang ML, Cui L, Zhao H, Zhao Y. 2015. E*ffect of nitrogen and phosphorus deficiency on transcriptional regulation of genes encoding key enzymes of starch metabolism in duckweed (Landoltia punctata)*. Plant Physiology and Biochemistry 86: 72-81.

Student Spotlight: Ms. Philomena Chu

Imagine biting into an apple. The sensation that you get from chomping into a crisp, sweet Fuji apple is quite different from biting into a mealy Red Delicious, or a tart Granny Smith. Despite the differences in texture and flavor, all three apples, including those varieties not mentioned here, are *Malus domestica* species. Each variety at the subspecies level may exhibit a distinct set of traits. The same holds true for duckweed. In my research, I focus on differentiating the varieties or strains of duckweed using molecular tools that exploit this genetic diversity for duckweed-based applications.

My team selected native, drought tolerant plants that would perform most robustly on a rooftop garden. I was intrigued by the observed phenotypic differences amongst these plants. On our green roof, why did some plants grow faster than others, and how were they able to achieve it? How could some plants withstand the harsh rooftop environment, while others would wither away?

Inspired by creating these unique landscapes, I was interested in studying plant biology to explore how plant science and human communities can lead to sustainable and productive interactions. However, I did not have the proper prerequisites to apply directly to graduate school from my first undergraduate degree in psychology, so I finished another bachelor's degree in plant science at Rutgers, and stayed for my Ph.D. with the goal of studying renewable energy. During a meeting early in my graduate studies, Dr. Eric Lam described the advantages of working with duckweed as a model organism and its application as a biofuel feedstock. My current research focuses broadly on duckweed as a renewable and sustainable source of energy. More specifically, once we find duckweed strains with our desired characteristics, how can we reliably and quickly tell them apart from less useful strains? Also, what are some of the molecular mechanisms underpinning duckweed biology and how can we use them to our advantage?

I was fortunate to be supported by a fellowship program from the National Science Foundation aimed at encouraging graduate students to work interdisciplinarily. The program also stressed the importance of international interdisciplinary collaborations. Among the many highlights was meeting Eduardo Mercovich and the other members of MamaGrande during trips to Argentina in November 2013 and then again in February 2015. There we toured multiple wastewater treatment facilities which collaborate with MamaGrande to treat municipal wastewater with duckweed—the most picturesque was PDLC Norte, an Aguas Del Norte site nestled in the mountains in the northwest province of Salta.

Scientific research should be accompanied by effective policy to produce large-scale impact. I recently interned at the New Jersey State Legislature in Trenton through a fellowship provided by the Eagleton Institute of Politics in order to gain first-hand experience of the way that government operates. I observed the arduous and dynamic decision-making process of passing a bill into law. Also, in addition to conducting policy research on behalf of legislators, I gained valuable insight into the laws and regulations relevant to the energy landscape at the state and national levels. For my career path, I intend to combine this knowledge with my scientific training to promote sustainable bioenergy that will benefit society both environmentally and economically.

I just finished a busy semester of juggling my internship at the New Jersey State Legislature with being a teaching assistant for a general biology course in addition to continuing my dissertation research. It has been an intellectually rewarding journey towards the completion of my Ph.D., which I will soon defend. I hope to continue exploring the mysteries of plants and to see what exciting duckweed and energy developments are in store!

Useful methods 6: LemnaTec Scanalyzer

a Versatile Tool for Duckweed Research and Testing

Marcus Jansen
Chief Scientist

Kevin Nagel
Application Scientist

LemnaTec GmbH, Pascalstraße 59, 52076 Aachen, Germany

Duckweed assessments

Duckweed growth inhibition tests are widely used for risk assessments in many areas. For instance, testing plant toxicity of chemicals, including plant protecting agents and other substances used in agriculture and gardening. Moreover such tests deliver ecotoxicological data on water and soil contamination.

The tests largely rely on repeated counting of frond numbers throughout the test period, in particular focusing on the change in frond development after application of the testing substance. Effects of testing substances on growth and development of fronds are evaluated in comparison to the development of untreated fronds growing in parallel.

How could LemnaTec help my routine work?

LemnaTec was founded in 1998, as a spin-off from RWTH Aachen, to develop a digital imaging system to automate frond counting in duckweed tests. Fronds, displayed on vessels with growth media typically used in duckweed assays, are imaged by the LemnaTec Scanalyzer platform. The resulting RGB images are automatically stored and analyzed by the LemnaTec's software that generates output tables containing all of the measured parameters. By repeating this procedure with multiple samples on subsequent days throughout the screening period, it is possible to monitor and compare temporal development of various treatments or sample-types. In particular, dynamic changes after applying testing materials to the duckweed can be monitored in a multi-factorial manner.

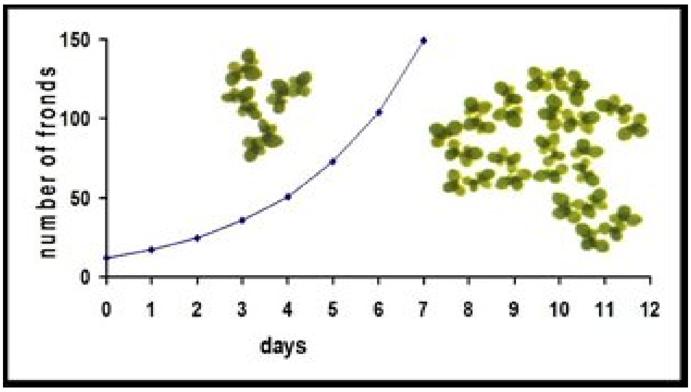

Figure 1 Example of automatically measured frond numbers over seven days.

Image processing tools to support duckweed measurements

LemnaGrid is a computer software package with a rich library of image processing functions (grid devices). It uses an intuitive approach to create image processing and image analysis algorithms by dragging and dropping functions from a library panel into the grid designer window, and then connecting the devices in a logical manner. The user, therefore, does not have to write lines of code but simply create workflows. Thereby, users can quickly create re-usable and customizable analysis procedures without the need to learn a programming language or involve data scientists.

A basic workflow consists of four main devices: an image processing module that is a database reader (DB Reader) together with a foreground/background color picker and an image analysis module that consists of a universal converter and a database writer (DB Writer).

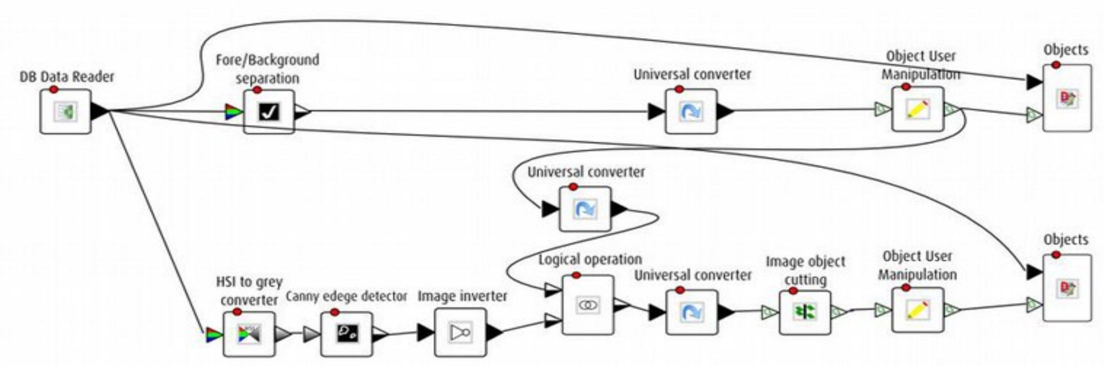

Figure 2: Example setup of a data processing pipeline for LemnaTec's image analysis built with the LemnaGrid software platform.

In this application example (Figure 2) digitized images of *Lemna* samples are loaded by the DB Reader. Due to a homogeneous background - in this case a white panel - the detection of image objects (image segmentation) can be efficiently done by defining white (R/G/B = 255/255/255 +/- 30) as the background. All other colors are taken as foreground. The result is a binary image (image mask) where white pixels represent the image object.

By converting all binary large objects (blobs) in the binary image into a list of objects with the Universal Converter, objects are counted and parameters for each object are determined, including its surface area (figure 3). Other morphometric parameters include center of mass, convex hull area, compactness, or bounding box dimensions such as width and height. The result of the analysis is written to the database with the DB Writer and can be retrieved from there for further analysis.

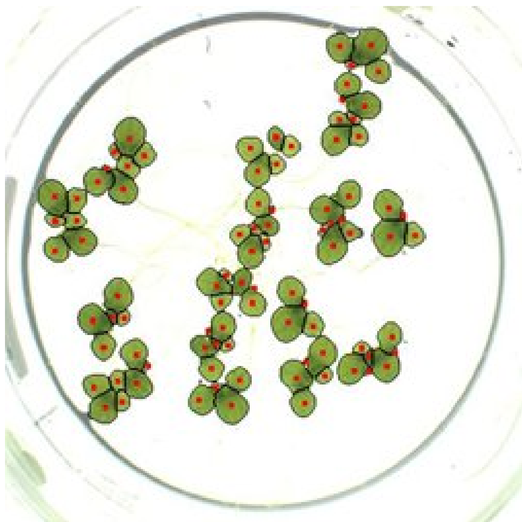

Figure 3: Duckweed fronds after analysis with LemnaGrid – red dots denote frond counts, single detected fronds are encircled by dark lines.

In order to determine the number of fronds, the processing pipeline converts the color image into an intensity image with the HSI to gray converter and uses the Canny Edge Detector to find edges between fronds. The result is used to modify the image mask with the Image Inverter and Logical Operator. In a next step the Image Object Cutter segments objects into smaller constituents, in this case *Lemna* fronds. Frond detection is a challenging task, because depending on the growth stage, age and physiology a clear edge between fronds may be hard to detect. Therefore the result is returned as a predicted solution to the user, and using the Object User Manipulation device, the user can refine the output.

Taken together, LemnaGrid enables an automated evaluation, but still leaves the possibility that users refine results in complex analysis cases.

Parameters derived from image processing

Image processing tools are more accurate and reproducible than visual inspection by human operators. Different from visual scoring that frequently delivers qualitative data, image processing enables capturing more quantitative parameters beyond the frond count. The processing pipeline can deliver numbers that describe sizes, shapes, and colors of the fronds in addition to the frond counts. These parameters link to biologically and eco-toxicologically relevant data and describe how phenotypic changes – in comparison to untreated healthy fronds – occur after exposure to toxic substances (Figure 4).

Frond counts, and particularly the change of them, are indicative of growth and multiplication processes, or if death occurred when the frond number decreased (Figure 1). Using image processing, the area of the fronds can be determined in order to assess growth more precisely than it is possible by just counting. The dynamics of the overall area of the visible fronds similarly link to growing or decreasing populations. In addition, area data on single fronds, and their distribution within the population, can reveal information on individual growth processes. As large numbers of small fronds might have the same overall area as small numbers of large fronds, it is important to combine parameters in order to comprehensively assess the developmental status of the fronds and their response to testing factors and substances.

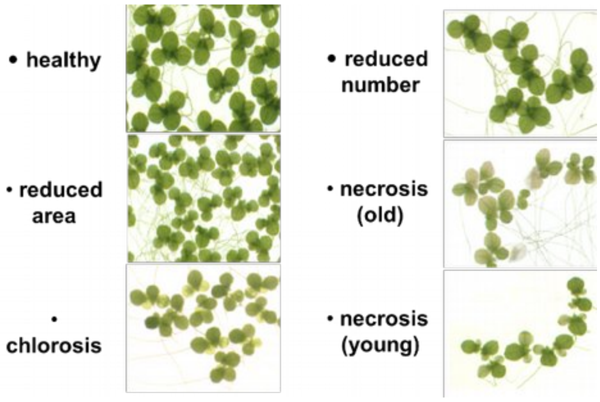

Figure 4 Typical phenotypic changes of stressed fronds in comparison to healthy fronds

Image analysis can provide information on surface colours, too, and thereby assist the estimation of whether fronds are healthy or stressed. Chlorosis and necrosis of frond evoke characteristic colour changes that can be quantified with image analysis tools. Such quantification enables precise calculation of the degree of chlorosis or necrosis and thereby helps to assess viability. Viability assessment in turn enables one to distinguish between living and dead fronds, which is important for rating the impact of testing substances on plant survival. Finally image analysis determines shape description that helps to detect stress-related shape changes.

In a pilot study, fronds were exposed to potassium dichromate in concentrations of 0.2 to 50 mg per liter of cultivation medium and compared to fronds growing without the testing substance (Figure 5). Already at the lowest concentration tested markedly diminished frond area can be observed, and the growth inhibition increased further with higher levels of potassium dichromate in the growth medium. Coincident with growth reduction, remaining frond area changed from healthy green to pale and yellow, indicating that large fractions of the remaining fronds undergone damage and were no longer viable. At control cultivation without toxins, a baseline of around 30% frond area having colour changes from green towards pale green and yellow were observed. This fraction grew to more than 75% at high concentrations of potassium dichromate. The results indicate that potassium

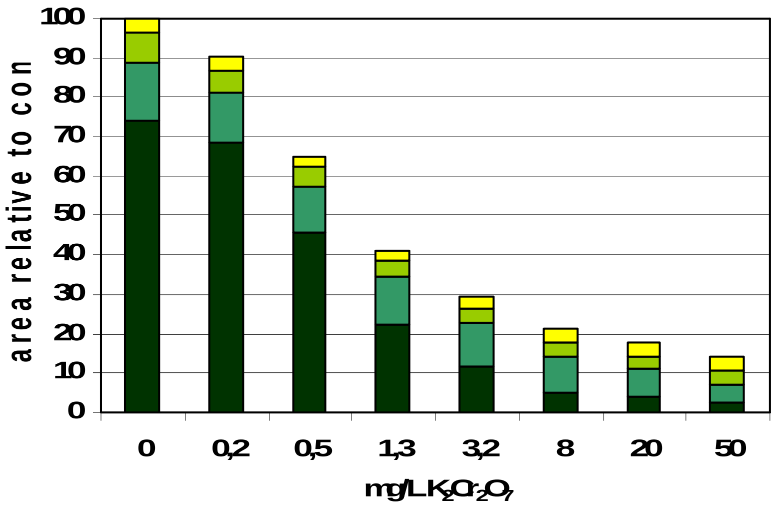

Figure 5: Frond area and color class fractions in dependency of increasing concentrations of a toxic substance (potassium dichromate).

dichromate not only inhibits the growth of new fronds, but also damages the existing fronds by reducing viable area.

Combining multiple duckweed characterizing factors derived from image processing together with biochemical data provides comprehensive sets of information in toxicology studies, for example in the assessment of phytotoxicity of cobalt ions by Sree et al. (2015).

Applications in research and monitoring

Duckweed assays are used in wastewater management in order to monitor whether substances with toxicity to plants remain after wastewater treatment processes. A research group in Lisbon analyzed the efficiency of wastewater cleaning with ecotoxicological test with different organisms, including *L. minor* (Mendonça et al., 2013). At different days throughout the week, and also at different times throughout the days, the observed toxicity of influent water changed due to wastewater composition. Primary and particularly secondary treatment steps in the cleaning procedure turned out to efficiently clean the water and eliminating toxic compounds. The results proved that such monitoring procedures are valuable tools for environmental management.

Nanoparticles are broadly used, e.g. for surface coatings or solar cells, but they are prone to have toxic effects on organisms. A research consortium in Lisbon, partly comprising the same researchers as the group mentioned in the previous paragraph, tested phytotoxicity of such particles using a *L. minor* assay (Picado et al., 2015). Although the tested particles were non-toxic to *L. minor*, there was toxicity to other organisms such as bacteria, algae, or crustaceae.

Chemical compounds of catalysts are potential sources of environmental pollution and damage; therefore ecotoxicological tests are meaningful assays to measure hazard potentials of such substances. A consortium of German and Polish researchers measured the toxic effect of Methyltrioxorhenium and derivatives thereof, which are suitable for use as catalysts in petrol chemistry (Stolte et al., 2015). Not only toxicity towards higher plants as determined with a Scanalzyer-assisted duckweed assay, but toxicity to various organisms led to the conclusion that preventing release of and exposure to such chemicals is essential.

Similarly, German and Polish researchers assessed ecotoxic properties of sweetener substances, again including a duckweed assay that should indicate toxicity of the tested substances towards plants (Stolte et al., 2013). As artificial sweeteners became popular as replacements for sugar, they occurred as anthropogenic trace substances in aquatic environments but their toxicity data are scarce. Assessing duckweed together with algae and water fleas, the researchers reported that common artificial sweeteners tested as well as the natural sweetener stevioside do not exhibit toxicity to such organisms, although additional data may be needed for more complete assessment.

Published literature on ecotoxicology studies relying on LemnaTec duckweed assays not only comprises these examples of water management, nanoparticle studies, catalyst assessment, and food additive characterisation, but there are examples available from herbicide testing (Grossmann and Ehrhardt, 2007; Tresch et al., 2008; Grossmann et al., 2012), natural compound assays (Meepagala et al., 2005; Diers et al., 2006; Cantrell et al., 2007; Cerdeira et al., 2012), homeopathic substance studies (Scherr et al., 2009; Jäger et al., 2010; Jäger et al., 2011) and other applications.

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From the Database

Highlights

The following two papers introduce a new phytotoxicity test with duckweeds:

Assessment of giant duckweed (*Spirodela polyrhiza* L. Schleiden) turions as model objects in ecotoxicological applications

Olah, V; Hepp, A; Meszaros, I

Bulletin of Environmental Contamination and Toxicology 96: 596-601 (2016)

In this study germination of *Spirodela polyrhiza* (L.) Schleiden (giant duckweed) turions was assessed under cadmium exposure to test applicability of a novel turion-based ecotoxicology method. Floating success of germinating turions, protrusion of the first and subsequent fronds as test endpoints were investigated and compared to results of standard duckweed growth inhibition tests with fronds of the same species. Our results indicate that turions can be used to characterize effects of toxic substances. Initial phase of turion germination (floating up and appearance of the first frond) was less sensitive to Cd treatments than the subsequent frond production. The calculated effective concentrations for growth rates in turion and normal frond tests were similar. Single frond area produced by germinating turions proved to be the most sensitive test endpoint. Single frond area and colony disintegration as additionally measured parameters in normal frond cultures also changed due to Cd treatments but the sensitivity of these parameters was lower than that of growth rates.

History and sensitivity comparison of the *Spirodela polyrhiza* microbiotest and Lemna toxicity tests

Baudo, R; Foudoulakis, M; Arapis, G; Perdaen, K; Lanneau, W; Paxinou, ACM; Kouvdou, S; Persoone, G

Knowledge and Management of Aquatic Ecosystems 416, Article Number: 23 (2015)

The history of toxicity tests with duckweeds shows that these assays with free-floating aquatic angiosperms are gaining increasing attention in ecotoxicological research and applications. Standard tests have been published by national and international organizations, mainly with the test species *Lemna minor* and *Lemna gibba*. Besides the former two test species the great duckweed *Spirodela polyrhiza* is to date also regularly used in duckweed testing. Under unfavourable environmental conditions, the latter species produces dormant stages (turions) and this has triggered the attention of two research groups from Belgium and Greece to jointly develop a "stock culture independent" microbiotest with *S. polyrhiza*. A 72 h new test has been worked out which besides its independence of stock culturing and maintenance of live stocks is very simple and practical to perform, and much less demanding in space and time than the conventional duckweed tests. Extensive International Interlaboratory Comparisons on the S. polyrhiza microbiotest showed its robustness and reliability and triggered the decision to propose this new assay to the ISO for endorsement and publication as a standard toxicity test for duckweeds. Sensitivity comparison of the 72 h *S. polyrhiza* microbiotest with the 7d L. minor assay for 22 compounds belonging to different groups of chemicals revealed that based on growth as the effect criterion both duckweed assays have a similar sensitivity. Taking into account its multiple advantages and assets, the *S. polyrhiza* microbiotest is a reliable and attractive alternative to the conventional duckweed tests.

Biotechnology

Succinic acid production from duckweed (*Landoltia punctata*) hydrolysate by batch fermentation of *Actinobacillus succinogenes* GXAS137

Shen, N; Wang, QY; Zhu, J; Qin, Y; Liao, SM; Li, Y; Zhu, QX; Jin, YL; Du, LQ; Huang, RB

Bioresource Technology 211: 307-312 (2016)

Duckweed is potentially an ideal succinic acid (SA) feedstock due to its high proportion of starch and low lignin content. Pretreatment methods, substrate content and nitrogen source were investigated to enhance the bioconversion of duckweed to SA and to reduce the costs of production. Results showed that acid hydrolysis was an effective pretreatment method because of its high SA yield. The optimum substrate concentration was 140 g/L. The optimum substrate concentration was 140 g/L. Corn steep liquor powder could be considered a feasible and inexpensive alternative to yeast extract as a nitrogen source. Approximately 57.85 g/L of SA was produced when batch fermentation was conducted in a 1.3 L stirred bioreactor. Therefore, inexpensive duckweed can be a promising feedstock for the economical and efficient production of SA through fermentation by *Actinobacillus succinogenes* GXAS137.

Computation-aided separation of seven components from *Spirodela polyrrhiza* (L.) via counter-current chromatography

Ren, DB; Han, BS; Xin, ZQ; Liu, WB; Ma, SS; Liang, YZ; Yi, LZ

Separation and Purification Technology 165: 160-165 (2016)

In this study, seven flavonoids were successfully separated from *Spirodela polyrrhiza* (L.) Schleid. via highspeed counter-current chromatography (HSCCC) with elution-extrusion and online recycling elution modes. The first step for successful HSCCC separation is the crucial solvent system selection. In this work, nonrandom two-liquid segment activity coefficient (NRTL-SAC) method, a computational strategy, was used to facilitate the solvent system selection process. According to the NRTL-SAC method, a suitable solvent system can be rapidly predicted and screened by calculating the partition coefficient rather than by performing tedious experimental measurements. Based on the method, two solvent systems of hexane/ethyl acetate/ethanol/water (1:3:1:3 and 1:9:1:9, v/v) were predicted as the most suitable systems for HSCCC separation. To avoid unnecessary waste of the stationary or mobile phase, both the mobile and stationary phases were prepared independently based on the calculated phase compositions by thermodynamic method. Consequently, seven compounds were successfully separated with high purity (>90%). The separated compounds were identified through UV spectra and high-resolution tandem mass spectroscopy. These compounds include orientin (1), vitexin (2), luteolin 7-O-glucoside (3), apigenin 7-O-glucoside (4), luteolin 8-C-(2 "-O-feruloyl-) glucoside (5), apigenin 8-C-(-2 "-O-feruloyl-) glucoside (6), and luteolin (7). This work demonstrates that HSCCC is suitable for separation of natural products, and computational strategy is very helpful for enhancing the efficiency of CCC experiments.

Starch characterization and ethanol production of duckweed and corn kernel

Lee, CJ; Yangcheng, HY; Cheng, JJ; Jane, JL

Starch-Staerke 68: 348-354 (2016)

The objectives of this study were to characterize the chemical structure, physical properties, enzyme digestibility, and ethanol production of duckweed starch and compare them with that of corn starch (B73). Duckweed consisted of 23.3% starch (dry basis, db), which was less than B73 corn kernels (66.5%). The morphology of duckweed starch granules displayed disk/dome shapes with one side of the granule flat and diameters of 4-9 mm. Duckweed starch displayed a B-type polymorph, having an average amylopectin branch-chain length of DP 26.5, and 35.7% amylose; both were larger than corn starch counterparts (DP 21.3 and 31.0%, respectively). Cooked duckweed starch showed higher resistant-starch content (14.1%) and a greater percentage of retrogradation after 7-day storage (57.5%) than the cooked B73 corn starch counterparts (8.7 and 49.6%, respectively). Duckweed plant produced a smaller ethanol-yield (12.0 g/100 g plant, db) but a greater ethanol-conversion efficiency (90.8%) than the B73 corn kernels (33.7 g/100 g kernels and 89.4%, respectively).

Ecology

Emission of Carbon Dioxide and Methane from Duckweed Ponds for Stormwater Treatment

Dai, JJ; Zhang, CQ; Lin, CH; Hu, ZQ

Water Environment Research 87: 805-812

This study determined the greenhouse gas emission from two laboratory-scale duckweed ponds for stormwater treatment. The rate of carbon dioxide (CO2) emission from the two duckweed systems was 1472 +/- 721 mg/m(2).d and 626 +/- 234 mg/m(2).d, respectively. After the removal of duckweeds, CO2 emissions decreased to 492 +/- 281 mg/m(2).d and 395 +/- 53 mg/m(2).d, respectively. The higher CO2 emissions in the duckweed systems were attributed to duckweed biomass decay on the pond soil surface. A thin-film model was able to predict the increasing CO2 concentrations in the closed static chamber during 2 weeks of sampling. The initial methane fluxes from the duckweed systems were 299 +/- 74 mg/m(2).d and 180 +/- 91 mg/m(2).d, respectively. After the removal of duckweeds, the flux increased to 559 +/- 215 mg/m(2).d and 328 +/- 114 mg/m(2).d, respectively.

Molecular Biology

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

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

New Phytologist 209 : 354-363 (2016)

Duckweeds are aquatic monocotyledonous plants of potential economic interest with fast vegetative propagation, comprising 37 species with variable genome sizes (0.158-1.88 Gbp). 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 0.89 Mbp 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.

Rapid and highly efficient callus induction and plant regeneration in the starch-rich duckweed strains of *Landoltia punctata*

Huang, M; Fu, LL; Sun, XP; Di, R; Zhang, JM

Acta Physiologiae Plantarum 38, Article Number: 122 (2016)

The starch-rich duckweed *Landoltia punctata* is a valuable aquatic plant in wastewater purification, bioenergy production, and many other applications. A highly efficient callus induction and plant regeneration protocol is desirable so that biotechnology can be used to develop new varieties with added value and adaptation. We studied both known and unknown factors that influence callus induction in *L. punctata* and obtained almost 100 % induction rate in 30 days. The optimum medium for callus induction was MS basal medium supplemented with 1 % sorbitol, 15 mg/L 2,4-D, and 2 mg/L 6-BA. Green fragile callus was induced from the meristematic region in the budding pouches. The optimum photoperiod for callus induction was 16-h day, and the optimum explant orientation was dorsal side down on the medium. The optimum medium for callus subculture was WPM basal medium supplemented with 2 % sorbitol, 4 mg/L 2,4-D, and 0.5 mg/L TDZ. Green callus could be maintained by subculture once every 4 weeks. However, when the subculture cycle was prolonged to 6 weeks or longer, yellow fragile embryogenic callus was obtained. The optimum plant regeneration medium was MS medium supplemented with 0.5 % sucrose, 1 % sorbitol, and 1.0 mg/L 6-BA with frond regeneration rates of approximately 90 %. The regenerated fronds rooted in Hoagland's liquid medium in 1 week. The callus induction and frond regeneration protocol was tested for its efficiency in geographically distinct strains 5502, 8721, and 9264. Thus, we obtained a rapid and efficient protocol for callus induction and frond regeneration of *L. punctata*, which takes only 9 weeks.

Transcriptomic and physiological analysis of common duckweed *Lemna minor* responses to NH4+ toxicity

Wang, WG; Li, R; Zhu, QL; Tang, XY; Zhao, Q

BMC Plant Biology 16: Article Number: 92 (2016)

Plants can suffer ammonium (NH4+) toxicity, particularly when NH4+ is supplied as the sole nitrogen source. However, our knowledge about the underlying mechanisms of NH4+ toxicity is still largely unknown. *Lemna minor*, a model duckweed species, can grow well in high NH4+ environment but to some extent can also suffer toxic effects. The transcriptomic and physiological analysis of *L. minor* responding to high NH4+ may provide us some interesting and useful information not only in toxic processes, but also in tolerance mechanisms. The *L. minor* cultured in the Hoagland solution were used as the control (NC), and in two NH4+ concentrations (NH4+ was the sole nitrogen source), 84 mg/L (A84) and 840 mg/L (A840) were used as stress treatments. The NH4+ toxicity could inhibit the growth of *L. minor*. Reactive oxygen species (ROS) and cell death were studied using stained fronds under toxic levels of NH4+. The malondialdehyde content and the activities of superoxide dismutase and peroxidase increased from NC to A840, rather than catalase and ascorbate peroxidase. A total of 6.62G nucleotides were generated from the three distinct libraries. A total of 14,207 differentially expressed genes (DEGs) among 70,728 unigenes were obtained. All the DEGs could be clustered into 7 profiles. Most DEGs were down-regulated under NH4+ toxicity. The genes required for lignin biosynthesis in phenylpropanoid biosynthesis pathway were up-regulated. ROS oxidative-related genes and programmed cell death (PCD)-related genes were also analyzed and indicated oxidative damage and PCD occurring under NH4+ toxicity.

The first large transcriptome study in *L. minor* responses to NH4+ toxicity was reported in this work. NH4+ toxicity could induce ROS accumulation that causes oxidative damage and thus induce cell death in *L. minor*. The antioxidant enzyme system was activated under NH4+ toxicity for ROS scavenging. The phenylpropanoid pathway was stimulated under NH4+ toxicity. The increased lignin biosynthesis might play an important role in NH4+ toxicity resistance.

GST (phi) gene from macrophyte *Lemna minor* is involved in cadmium exposure responses

Chen, SH; Chen, X; Dou, WH; Wang, L; Yin, HB; Guo, SL

Chinese Journal of Oceanology and Limnology 34: 342-351 (2016)

Reactive oxygen species (ROS) scavengers, including ascorbate peroxidase, superoxide dismutase, catalase and peroxidase, are the most commonly used biomarkers in assessing an organisms' response to many biotic and abiotic stresses. In this study, we cloned an 866 bp GST (phi) gene in *Lemna minor* and investigated its characteristics, expression and enzymatic activities under 75 mu mol/L cadmium concentrations in comparison with other ROS scavengers. GST (phi) gene expression patterns were similar to those of other scavengers of ROS. This suggests that GST (phi) might be involved in responding to heavy metal (cadmium) stress and that its expression level could be used as a bio-indicator in monitoring cadmium pollution.

Interaction with other organisms

Quantification and enzyme targets of fatty acid amides from duckweed root exudates involved in the stimulation of denitrification

Sun, L; Lu, Y; Kronzucker, HJ; Shi, W

Journal of Plant Physiology 198: 81-88 (2016)

Fatty acid amides from plant root exudates, such as oleamide and erucamide, have the ability to participate in strong plant-microbe interactions, stimulating nitrogen metabolism in rhizospheric bacteria. However, mechanisms of secretion of such fatty acid amides, and the nature of their stimulatory activities on microbial metabolism, have not been examined. In the present study, collection, pre-treatment, and determination methods of oleamide and erucamide in duckweed root exudates are compared. The detection limits of oleamide and erucamide by gas chromatography (GC) (10.3ngmL(-1) and 16.1ngmL(-1), respectively) are shown to be much lower than those by liquid chromatography (LC) (1.7 and 5.0mugmL(-1), respectively). Quantitative GC analysis yielded five times larger amounts of oleamide and erucamide in root exudates of *Spirodela polyrrhiza* when using a continuous collection method (50.20±4.32 and 76.79±13.92mugkg(-1) FW day(-1)), compared to static collection (10.88±0.66 and 15.27±0.58mugkg(-1) FW day(-1)). Furthermore, fatty acid amide secretion was significantly enhanced under elevated nitrogen conditions (>300mgL(-1)), and was negatively correlated with the relative growth rate of duckweed. Mechanistic assays were conducted to show that erucamide stimulates nitrogen removal by enhancing denitrification, targeting two key denitrifying enzymes, nitrate and nitrite reductases, in bacteria. Our findings significantly contribute to our understanding of the regulation of nitrogen dynamics by plant root exudates in natural ecosystems.

Determination of the time-dependent response of *Lemna trisulca* to the harmful impact of the cyanotoxin anatoxin-a

Kaminski, A; Chrapusta, E; Adamski, M; Bober, B; Zabaglo, K; Bialczyk, J

Algal Research-Biomass Biofuels and Bioproducts 16: 368-375 (2016)

Previous studies reported anatoxin-a (ANTX-a) accumulation and bioremediation by the aquatic macrophyte *Lemna trisulca*. In the present study, we determined that cultivation of this macrophyte in medium containing ANTX-a at a concentration similar to that occur in nature (1.0 mu g.mL(-1)) did not cause any changes in the plant physiology. A much higher toxin concentration (12.5 mu g.mL(-1)) reduced photosynthetic efficiency by 27% compared with the control, but had no significant effect on the respiration process. *L. trisulca* cultivated in medium containing 25.0 mu g of ANTX-a.mL(-1) reduced the chl(a) content by 40% on the 18th day of the experiment, the chl(b) content by 35% on the 14th day of the experiment and the total carotenoids by 11% on the 24th day of the experiment. ANTX-a at concentrations <= 12.5 mu g.mL(-1) did not cause any significant differences in the concentration of released anions (Cl, NO3 and SO42) compared to the control. However, cultivation of *L. trisulca* with 5 mu g of ANTX-a.mL(-1) resulted in a 1.8-fold increase in the concentration of K+ released into the medium compared with the control. The amount of Na+, Ca2+ and Mg2+ released after plant exposure to 12.5 mu g of ANTX-a.mL(-1) was 1.3-fold, 1.6-fold and 1.7-fold higher, respectively, compared to the control. ANTX-a had a slight effect on the protein content and the oxidoreductase activity in the macrophyte. After 24 h, there were no significant differences in the enzymatic activity of the superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase and glutathione reductase, regardless of the ANTX-a concentrations in the media. Only polyphenol oxidase showed a slight increase in the activity in media containing ANTX-a. These findings confirmed that *L. trisulca* has a high tolerance to this toxin and a great potential as a phytoremediation agent in the aquatic environments.

Hiding in plain sight: *Koshicola spirodelophila* gen. et sp nov (Chaetopeltidales, Chlorophyceae), a novel green alga associated with the aquatic angiosperm *Spirodela polyrhiza*

Watanabe, S; Fucikova, K; Lewis, LA; Lewis, PO

American Journal of Botany 103: 865-875 (2016)

Discovery and morphological characterization of a novel epiphytic aquatic green alga increases our understanding of Chaetopeltidales, a poorly known order in Chlorophyceae. Chloroplast genomic data from this taxon reveals an unusual architecture previously unknown in green algae. Using light and electron microscopy, we characterized the morphology and ultrastructure of a novel taxon of green algae. Bayesian phylogenetic analyses of nuclear and plastid genes were used to test the hypothesized membership of this taxon in order Chaetopeltidales. With next-generation sequence data, we assembled the plastid genome of this novel taxon and compared its gene content and architecture to that of related species to further investigate plastid genome traits. The morphology and ultrastructure of this alga are consistent with placement in Chaetopeltidales (Chlorophyceae), but a distinct trait combination supports recognition of this alga as a new genus and species-*Koshicola spirodelophila* gen. et sp. nov. Its placement in the phylogeny as a descendant of a deep division in the Chaetopeltidales is supported by analysis of molecular data sets. The chloroplast genome is among the largest reported in green algae and the genes are distributed on three large (rather than a single) chromosome, in contrast to other studied green algae. The discovery of *Koshicola spirodelophila* gen. et sp. nov. highlights the importance of investigating even commonplace habitats to explore new microalgal diversity. This work expands our understanding of the morphological and chloroplast genomic features of green algae, and in particular those of the poorly studied Chaetopeltidales.

Physiology

A multigenerational effect of parental age on offspring size but not fitness in common duckweed (*Lemna minor*)

Barks, PM; Laird, RA

Journal of Evolutionary Biology 29: 748-756 (2016)

Classic theories on the evolution of senescence make the simplifying assumption that all offspring are of equal quality, so that demographic senescence only manifests through declining rates of survival or fecundity. However, there is now evidence that, in addition to declining rates of survival and fecundity, many organisms are subject to age-related declines in the quality of offspring produced (i.e. parental age effects). Recent modelling approaches allow for the incorporation of parental age effects into classic demographic analyses, assuming that such effects are limited to a single generation. Does this single-generation' assumption hold? To find out, we conducted a laboratory study with the aquatic plant *Lemna minor*, a species for which parental age effects have been demonstrated previously. We compared the size and fitness of 423 laboratory-cultured plants (asexually derived ramets) representing various birth orders, and ancestral birth-order genealogies'. We found that offspring size and fitness both declined with increasing immediate' birth order (i.e. birth order with respect to the immediate parent), but only offspring size was affected by ancestral birth order. Thus, the assumption that parental age effects on offspring fitness are limited to a single generation does in fact hold for *L. minor*. This result will guide theorists aiming to refine and generalize modelling approaches that incorporate parental age effects into evolutionary theory on senescence.

Phytomedicine

Improvement of atopic dermatitis with topical application of *Spirodela polyrhiza*

Lee, HJ; Kim, MH; Choi, YY; Kim, EH; Hong, J; Kim, K; Yang, WM

Journal of Ethnopharmacology 180: 12-17 (2016)

Ethnopharmacological relevance: *Spirodela polyrhiza* has been used as a traditional remedy for the treatment of urticarial, acute nephritis, inflammation, as well as skin disease.

Atopic dermatitis (AD) is characterized hyperplasia of skin lesion and increase of serum immunoglobulin E (IgE) level. In this study, the topical effects of S. polyrhiza (SP) on 2, 4-dinitro-chlorobenzene (DNCB)-induced AD mice model were investigated by several experiments. BALB/c mice were randomly divided into five groups as NOR, CON, DEX, SP 1, and SP 100 groups (n=5, respectively). To induce atopic dermatitis-like skin lesions, DNCB had been applied on shaved dorsal skin. SP was topically treated to DNCB-induced mice as 1 and 100 mg/mL concentrations. Histological changes were showed by hematoxylin and eosin (H&E) staining and the infiltration of mast cells was detected by toluidine blue staining. In addition, the level of IgE and each cytokines were measured and expressions of inflammatory signaling factors were analyzed by western blotting assay. SP treatment improved a hyperplasia of epidermis and dermis in DNCB-induced AD-like skin lesion. The infiltration of mast cells was also decreased by treatment of SP. In addition, SP reduced the level of IgE in serum and attenuated the secretion of cytokines such as interleukin (IL)-4, IL-6, and tumor necrosis factor (TNF)-alpha. Treatment of SP also inhibited the expressions of pro-inflammatory mediators including nuclear factor-kappa B (NE-kappa B), phosphor-I kappa B-alpha, and mitogen-activated protein kinase (MAPK)s. From these data, we propose that SP ameliorates AD via modulation of pro-inflammatory mediators. SP may have the potential to be used as an alternative for treatment of AD.

Phytoremediation

Land spreading of sewage sludge in forest plantations: effects on the growth of the duckweed *Lemna minor* and trace metal bioaccumulation in the snail *Cantareus aspersus*

Mohamed, B; Frederic, G; Laurence, A-S; Pierre-Marie, B; Badr, A-S; Lotfi, A

Environmental Science and Pollution Research International 23: 9891-9900 (2016)

Wastewater plants generated annually millions of tons of sewage sludge (SS). Large amounts of this organic residue are spread on agricultural lands as a fertilizer, although it is viewed as a major potential source of contamination, presenting a danger to the terrestrial and aquatic environments. This study was undertaken to evaluate the impact of this practice on the duckweed *Lemna minor* and the snail *Cantareus aspersus*. Sludge was applied to soil either at six different loading rates equal to 0, 0.4, 3, 10, 30, and 60tons dry matter (DM)ha(-1) for *L. minor* test or at three rates equal to 0, 30, and 60tons DMha(-1) for C. aspersus test. At the highest rate of SS application (60tons DMha(-1)), the eluates showed that an increase in pH (6.1) resulted in a decrease in Al levels. Thus, the high stimulation of *L. minor* growth observed after this high rate of SS application can be explained by (i) a reduction in Al toxicity after precipitation and (ii) macro- and micronutrient enrichment. At a rate of SS application of only 30tons DMha(-1), growth appeared to be slightly significant ($p<0.05$), in spite of the significant increase in essential mineral elements. However, it is very difficult to discriminate between Al toxicity and pH effects. For the test with *C. aspersus*, the snail biomass was not affected by sludge application over the exposure period. Mortality was extremely low, with a rate of less than 4% at the last sampling date. Yet, Cu, Pb, and Cd accumulated significantly in the soft body of snails exposed to SS application, suggesting that the amount of metals excreted is lower than that absorbed. In contrast, Zn levels remain constant, inferring that absorption and elimination of Zn are balanced at the beginning of the experiment.

Phytoremediation of Drainage Water Containing Mono Ethylene Glycol Using a Duckweed (*Lemna gibba*) Pond System

Allam, A; Tawfik, A; Yoshimura, C

Journal of Environmental Engineering 142, Article Number: 04016014 (2016)

This study investigated the treatment of drainage water containing mono ethylene glycol (MEG) using duckweed (*Lemna gibba*). Six identical continuous flow duckweed-based treatment systems (DBS) were used at different initial MEG concentrations and hydraulic retention times (HRTs). The MEG removal efficiency decreased from 85.9 +/- 1.3 to 79.3 +/- 2.1% by increasing the initial concentration from 200 to 400mgMEG/L, respectively. However, the efficiency of DBS deteriorated significantly at higher MEG concentrations; i.e., the removal efficiencies decreased to 36.4 +/- 1.2% and 7.1 +/- 0.9% at concentrations of 800 and 1,200mgMEG/L, respectively. The performance of DBS was completely inhibited at MEG concentrations exceeding 1,200mg/L. The removal efficiency of MEG significantly increased from 37.2 +/- 1.3 to 79.3 +/- 2.1% when HRT was increased from 6 to 18days. Nevertheless, the removal efficiencies of MEG remained unaffected when HRT was increased up to 21days. The uptake process of MEG by duckweed typically followed first-order exponential decay relationships. These results support the recommendation that a duckweed pond system be operated at an HRT of 18days, and with a MEG concentration below 400mg/L.

Bioaccumulation of tetracycline and degradation products in *Lemna gibba L.* exposed to secondary effluents

Topal, M; Senel, GU; Obek, E; Topal, EIA

Desalination and Water Treatment 57: 8270-8277 (2016)

The aim of this study was determination of the bioaccumulation capacity of tetracycline (TC) and the degradation products by *Lemna gibba L.* in the pilot-scale reactor that takes the effluent of a municipal wastewater treatment plant (Elazig city, Turkey). For this aim, *L. gibba L.* which was exposed to secondary clarifier effluent of the treatment plant was harvested from the pilot-scale reactor. Then, the harvested *L. gibba L.* was extracted and passed from solid-phase extraction cartridge and TC, 4-epitetracycline (ETC), 4-epianhydrotetracycline (EATC), and anhydrotetracycline (ATC) concentrations were determined. Maximum TC, ETC, EATC, and ATC concentrations bioaccumulated by *L. gibba L.* were 123 +/- 2.0, 129 +/- 3.2, 42.7 +/- 0.5, and 31.9 +/- 0.3ppb, respectively while minimum TC, ETC, EATC, and ATC concentrations those bioaccumulated by *L. gibba L.* were determined as 99.7 +/- 1.2, 111 +/- 2.2, 12 +/- 0.6, and 8.3 +/- 0.1ppb, respectively. The order of the uptake rate of TC and the degradation products by *L. gibba L.* was determined as follows: ETC>TC>EATC>ATC.

Lead and cadmium removal from water using duckweed - *Lemna gibba L.*: Impact of pH and initial metal load

Verma, R; Suthar, S

Alexandria Engineering Journal 54: 1297-1304 (2015)

The aim of this study was to investigate the potential of duckweed (*Lemna gibba*) in heavy metal (Pb and Cd) from water under different pH and metal loads. A total of three (2, 5 and 10 mg/L) strengths of Pb and Cd were used with varying pH (5, 7 and 9) and changes in metal concentration and metal uptake yield of system were recorded. The Pb and Cd removal ranged between 60.1% (2 mg/L at 9 pH) and 98.1% (10 mg/L at 7 pH) and 41.6% (10 mg/L at pH 9) and 84.8% (2 mg/L at pH 7), respectively. The duckweed set-up with pH 7 showed the optimum metal removal. The metal removal rate showed an inverse relationship with pH (r(2) > 0.60, for all). Bioconcentration factor (BCF) and metal uptake yield per unit of dry biomass (q(m)) were recorded: 403-738 and 445-616, respectively for BCFPb and BCFCd. The q(m) suggest the dose (mg/L) 5 and 10 at pH 5 as the best combinations for the optimum removal. Results, thus suggest that *L. gibba* can be a suitable candidate for removal of heavy metals from pollutant water bodies.

Bioaccumulation of copper, cadmium and nickel in duckweed (*Lemna trisulca*)

Kara, Y; Akba, O

Fresenius Environmental Bulletin 24: 3479-3482 (2015)

Duckweeds are small free-floating plants often farming dense mats on the surface of still or slow flowing water. *Lemna trisulca* (Duckweed) was examined for its ability to remove heavy metals. In laboratory conditions, plants were exposed to the copper, cadmium and nickel concentrations of 1.0, 3.0, 5.0 and 7.0 mg L-1 in certain periods (24, 48, 72 and 96 hours). The accumulation levels of metals in plants depending on different time and concentrations were investigated by using atomic absorption spectrometer. The results on bioaccumulation rates of Ni, Cd and Cu ions in Duckweed showed that the plant was able to remove the heavy metals. The maximum BCF values obtained were 541 for Cd, 848 for Cu and 1867 for Ni. Among the metals used, Ni was found to be accumulated at higher levels depending on the metal concentrations and time periods.

Potentials of using duckweed (*Lemna gibba*) for treatment of drainage water for reuse in irrigation purposes

Allam, A.; Tawfik, A.; El-Saadi, A.; Negm, A

Desalination and Water treatment 57: 459-467 (2016)

The potential use of duckweed (*Lemna gibba*) for the treatment of drainage water was investigated. Three continuous flow duckweed-based treatment systems (one-pond, two-pond, and three-pond) were used. Removal efficiencies of COD total and ammonia in the two-pond system were significantly higher (60.2 +/- 6.1% and 80.2 +/- 1.4%) than that found for single-pond system (30.6 +/- 7.9% and 56.8 +/- 3.3%), respectively, at a total hydraulic retention time (HRT) of 14d. Performance of three-pond system connected in series was evaluated at different HRTs of 21, 14, and 7d. Results showed that increasing the HRT and area of duckweed pond to pond depth (A(duckweed)/d(pond)) ratio from 7 to 14d and from 63.83 to 127.66 substantially increased the removal efficiency of COD total from 59.7 +/- 3.29 to 88.34 +/- 1.82%, respectively, resulting an effluent quality of 13.6 +/- 2.3 mg COD/L in the treated effluent. However, the removal efficiency of COD total remained almost constant when increasing the HRT from 14 to 21d and A(duckweed)/d(pond) from 127.66 to 191.49. This was not the case for nitrification efficiency, where ammonia removal increased from 32.6 +/- 7.95 to 71.75 +/- 6.1% and from 71.75 +/- 6.1 to 85.6 +/- 4.6% when increasing the HRT from 7 to 14d and from 14 to 21d, respectively.

Phytotoxicity

Duckweeds for water remediation and toxicity testing (Review)

Ziegler P; Sree KS; Appenroth KJ

Toxicological & Environmental Chemistry 2016. http://dx.doi.org/10.1080/02772248.2015.1094701 (2016)

The presence of toxic substances in wastewaters and outdoor bodies of water is an important ecotoxicological issue. The aim of this review is to illustrate how duckweeds, which are small, simply constructed, floating aquatic plants, are well suited to addressing this concern. The ability of duckweeds to grow rapidly on nutrient-rich water and to facilitate the removal of many substances from aqueous solution comprises the potential of these macrophytes for the remediation of wastewater and polluted aqueous reservoirs, while producing usable biomass containing the unwanted substances having been taken up. Their ease of cultivation under controlled and even sterile conditions makes duckweeds excellent test organisms for determining the toxicity of water contaminants, and duckweeds are important as model aquatic plants in the assessment of ecotoxicity. Duckweeds are also valuable for establishing biomarkers for the toxic effects of water contaminants on aquatic higher plants, but the current usefulness of duckweed biomarkers for identifying toxicants is limited. The recent sequencing of a duckweed genome holds the promise of combining the determination of water contaminant toxicity with toxicant diagnostics by means of gene expression profiling via DNA microarrays.

Assessing Toxicity of Organic Aquatic Micropollutants Based on the Total Chlorophyll Content of *Lemna minor* as a Sensitive Endpoint

Fekete-Kertesz, I; Kunglne-Nagy, Z; Gruiz, K; Magyar, A; Farkas, E; Molnar, M

Periodica Polytechnica-Chemical Engineering 59: 262-271 (2015)

The present study examined the chlorophyll content in a 7-day contact time experiment series. *Lemna minor* was exposed to caffeine, benzophenone, bisphenol A, 3,4-dichlorophenol, metamizole-Na, Na-diclofenac, acetochlor, atrazine, diuron, metazachlor and metolachlor to find a convenient sensitive response to the tested chemicals including some emerging micropollutants. The results demonstrated the differences in sensitivity to the tested micropollutants. As anticipated the industrial chemicals and the pesticides were the most toxic. The lowest observed effect concentration (LOEC) values determined for 3,4-dichlorophenol, acetochlor, diuron, metazachlor and metolachlor were 2.5 mu g/L, 0.05 mu g/L, 0.5 mu g/L, 5 mu g/L and 0.5 mu g/L, respectively. These values were comparable with the environmental concentrations reported in literature. Our study provides valuable information on the feasibility of *Lemna minor* total chlorophyll method as a sensitive and reliable bioassay for testing toxicity at mu g/L range and it may support risk assessment of organic micropollutants in freshwater ecosystems.

Molecular distribution and toxicity assessment of praseodymium by *Spirodela polyrrhiza*

Xu, T; Su, C; Hu, D; Li, F; Lu, Q; Zhang, T; Xu, Q

Journal of Hazardous Materials 312: 132-40 (2016)

Aquatic macrophytes are known to accumulate and bioconcentrate metals. In this study, the physiological, biochemical, and ultrastructural responses of *Spirodela polyrrhiza* to elevated concentrations of praseodymium (Pr), ranging from 0 to 60muM, were investigated over 20 d exposure. The results showed that the accumulation of Pr in *S. polyrrhiza* occurred in a concentration-dependent manner. The accumulation of Pr in biomacromolecules decreased in the order of cellulose and pectin (65-69%), crude proteins (18-25%), crude polysaccharides (6-10%), crude lipids (3%-4%). Significant increases in malondialdehyde (MDA), and decreases in photosynthetic pigment, soluble protein, and unsaturated fatty acids showed that Pr induced oxidative stress. Inhibitory effects on photosystem II and the degradation of the reaction center proteins D1 and D2 were revealed by chlorophyll a fluorescence transients, immunoblotting, and damage to chloroplast ultrastructure. Significant increases in cell death were observed in Pr-treated plants. However, *S. polyrrhiza* can combat Pr induced oxidative injury by activating various enzymatic and non-enzymatic antioxidants. These results will improve understanding of the

biological consequences of rare earth elements (REEs) contamination, particularly in aquatic bodies.

The different response mechanisms of *Wolffia globosa*: Light-induced silver nanoparticle toxicity

Zou, X; Li, P; Huang, Q; Zhang, H

Aquatic Toxicology 176: 97-105 (2016)

Silver nanoparticles (AgNPs) have emerged as a promising bactericide. Plants are a major point of entry of contaminants into trophic chains. Here, the physiological responses of *Wolffia globosa* to AgNPs have been probed using different light schemes, and these data may reveal new insights into the toxic mechanism of AgNPs. *W. globosa* was grown in culture medium and treated with different concentrations of AgNPs for 24h under pre- and post-illuminated conditions. However, fluorescence quenching, the accumulation of sugar and the reduction of Hill reaction activity were found in response to the AgNP-stresses. In the pre-illuminated condition, oxidative damage was obvious, as indicated by the higher malondialdehyde (MDA) content and an up-regulation of superoxide dismutase (SOD) activity. The maximum increases of MDA content and SOD activity were 1.14 and 2.52 times the respective controls when exposed to 10mg/L AgNPs. In contrast, in the post-illuminated condition, the alterations in photosynthetic pigment and soluble proteins content were more significant than the alterations in oxidative stress. The contents of chlorophyll a, carotenoids and soluble protein decreased to 77.7%, 66.2% and 72.9% of the controls after treatment with the highest concentration of AgNPs (10mg/L). Based on the different physiological responses, we speculated that in the pre-illuminated condition, oxidative stress was responsible for the decline in the oxygen evolution rate, while in the post-illuminated condition, the decrease in the Hill reaction activity could be attributed to the blocking of electron transfer and an insufficient proton supply. Our findings demonstrate that environmental factors regulate the physiological responses of plants to AgNPs through distinct mechanisms.

The first toxicological study of the antiozonant and research tool ethylene diurea (EDU) using a *Lemna minor L.* bioassay: Hints to its mode of action

Agathokleous, E; Mouzaki-Paxinou, A-C; Saitanis, CJ; Paoletti, E; Manning, WJ

Environmental Pollution 213: 996-1006 (2016)

The antiozonant and research tool ethylene diurea (EDU) is widely studied as a phytoprotectant against the widespread pollutant ground-surface ozone. Although it has been extensively used, its potential toxicity in the absence of ozone is unknown and its mode of action is unclear. The purpose of this research was to toxicologically assess EDU and to further investigate its mode of action using *Lemna minor L.* as a model organism. Application of EDU concentrations greater than 593mgL(-1) (practically 600mgL(-1)) resulted in adverse inhibition of colony growth. As no-observed-toxic-effects concentration (NOEL) we recommend a concentration of 296mgL(-1) (practically 300mgL(-1)). A hormetic response was detected, i.e. stimulatory effects of low EDU concentrations, which may indicate overcompensation in response to disruption in homeostasis. Growth inhibition and suppressed biomass were associated with impacted chlorophyll a fluorescence (PhiPSII, qP and ETR). Furthermore, EDU increased mesophyll thickness, as indicated by frond succulence index. Applications of concentrations ≥593mgL(-1) to uncontrolled environments should be avoided due to potential toxicity to sensitive organisms and the environment.

Response of *Lemna minor L.* to short-term cobalt exposure: The effect on photosynthetic electron transport chain and induction of oxidative damage

Begovic, L; Mlinaric, S; Antunovic Dunic, J; Katanic, Z; Loncaric, Z; Lepedus, H; Cesar, V

Aquatic Toxicology 175:117-126 (2016)

The effect of two concentrations of cobalt (Co(2+)) on photosynthetic activity and antioxidative response in *Lemna minor L.* were assessed 24, 48 and 72h after the start of the exposure. Higher concentration of cobalt (1mM) induced growth inhibition while lower concentration (0.01mM) increased photosynthetic pigments content. Analysis of chlorophyll a fluorescence transients revealed high sensitivity of photosystem II primary photochemistry to excess of Co(2+) especially at the higher concentration where decreased electron transport beyond primary quinone acceptor QA(-) and impaired function of oxygen evolving complex (OEC) was observed. Due to impairment of OEC, oxygen production was decreased at higher Co(2+) concentration. Activity of superoxide dismutase was mainly inhibited while lipid peroxidation increased, at both concentrations, indicating that cobalt-induced oxidative damage after short exposure and moreover, susceptibility of the membranes in the cell to cobalt toxicity. Results obtained in this study suggest possible application of used parameters as tools in assessment of early damage caused by metals.

Modelling algae-duckweed interaction under chemical pressure within a laboratory microcosm

Lamonica, D; Clement, B; Charles, S; Lopes, C

Ecotoxicology and Environmental Safety 128: 252-265 (2016)

Contaminant effects on species are generally assessed with single-species bioassays. As a consequence, interactions between species that occur in ecosystems are not taken into account. To investigate the effects of contaminants on interacting species dynamics, our study describes the functioning of a 2-L laboratory microcosm with two species, the duckweed *Lemna minor* and the microalgae *Pseudo-kirchneriella subcapitata*, exposed to cadmium contamination. We modelled the dynamics of both species and their interactions using a mechanistic model based on coupled ordinary differential equations. The main processes occurring in this two-species microcosm were thus formalised, including growth and settling of algae, growth of duckweeds, interspecific competition between the two species and cadmium effects. We estimated model parameters by Bayesian inference, using simultaneously all the data issued from multiple laboratory experiments specifically conducted for this study. Cadmium concentrations ranged between 0 and 50 mu g.L-1. For all parameters of our model, we obtained biologically realistic values and reasonable uncertainties. Only duckweed dynamics was affected by interspecific competition, while algal dynamics was not impaired. Growth rate of both species decreased with cadmium concentration, as well as competition intensity showing that the interspecific competition pressure on duckweed decreased with cadmium concentration. This innovative combination of mechanistic modelling and model guided experiments was successful to understand the algae-duckweed microcosm functioning without and with contaminant. This approach appears promising to include interactions between species when studying contaminant effects on ecosystem functioning.

Combined effects of elevated CO2 and Cd-contaminated water on growth, photosynthetic response, Cd accumulation and thiolic components status in *Lemna minor L.*

Pietrini, F; Bianconi, D; Massacci, A; Iannelli, MA

Journal of Hazardous Materials 309: 77-86 (2016)

The objective of this study was to investigate the combined effects of elevated CO2 and cadmium (Cd) treatments on growth, photosynthetic efficiency and phytoremediation ability in *Lemna minor L.* Plants of L. minor were exposed to different Cd concentrations (0,1.5, 2.5 and 5 mg L-1 Cd) for periods of 24, 48 and 72 h at ambient (AC) and at elevated (EC) CO2 (350 and 700 ppm, respectively). Cadmium concentration, bioconcentration factor, enzyme activities and thiols content enhanced in plants with the increase of Cd treatments, time of exposure and at both CO2 levels. Glutathione levels increased only at AC. Growth, photosynthetic and chlorophyll fluorescence parameters, and the reduced glutathione to oxidized glutathione ratio declined in plants with increasing exposure time, Cd treatments and at both CO2 levels. Our results suggested that the alleviation of toxicity, at low Cd doses, observed in *L. minor* grown at EC is dependent on both increased photosynthesis and an enhanced antioxidant capacity.

The toxicity of zinc oxide nanoparticles to *Lemna minor (L.)* is predominantly caused by dissolved Zn

Chen, XL; O'Halloran, J; Jansen, MAK

Aquatic Toxicology 174: 46-53 (2016)

Nano-ZnO particles have been reported to be toxic to many aquatic organisms, although it is debated whether this is caused by nanoparticles per se, or rather dissolved Zn. This study investigated the role of dissolved Zn in nano-ZnO toxicity to *Lemna minor*. The technical approach was based on modulating nano-ZnO dissolution by either modifying the pH of the growth medium and/or surface coating of nano-ZnO, and measuring resulting impacts on *L. minor* growth and physiology. Results show rapid and total dissolution of nano-ZnO in the medium (pH 4.5). Quantitatively similar toxic effects were found when *L. minor* was exposed to nano-ZnO or the "dissolved Zn equivalent of dissolved nano-ZnO". The conclusion that nano-ZnO toxicity is primarily caused by dissolved Zn was further supported by the observation that phytotoxicity was absent on medium with higher pH-values (>7), where dissolution of nano-ZnO almost ceased. Similarly, the reduced toxicity of coated nano-ZnO, which displays a slower Zn dissolution, is also consistent with a major role for dissolved Zn in nano-ZnO toxicity.

The impact of humic acid on chromium phytoextraction by aquatic macrophyte *Lemna minor*

Kalcikova, G; Zupancic, M; Jemec, A; Gotvajn, AZ

Chemosphere 147: 311-317 (2016)

Studies assessing chromium phytoextration from natural waters rarely consider potential implications of chromium speciation in the presence of ubiquitous humic substances. Therefore, the present study investigated the influence of environmentally relevant concentration of humic acid (TOC = 10 mg L-1) on chromium speciation (Cr = 0.15 mg L-1) and consequently on phytoextraction by aquatic macrophyte duckweed *Lemna minor*. In absence of humic acid, only hexavalent chromium was present in water samples and easily taken up by *L. minor*. Chromium uptake resulted in a significant reduction of growth rate by 22% and decrease of chlorophyll a and chlorophyll b contents by 48% and 43%, respectively. On the other hand, presence of humic acid significantly reduced chromium bioavailability (57% Cr uptake decrease) and consequently it did not cause any measurable effect to duckweed. Such effect was related to abiotic reduction of hexavalent chromium species to trivalent. Hence, findings of our study suggest that presence of humic acid and chromium speciation cannot be neglected during phytoextraction studies.

Cyto-histological and morpho-physiological responses of common duckweed (*Lemna minor L.*) to chromium

Reale, L; Ferranti, F; Mantilacci, S; Corboli, M; Aversa, S; Landucci, F; Baldisserotto, C; Ferroni, L; Pancaldi, S; Venanzoni, R

Chemosphere 145: 98-105 (2016)

Along with cadmium, lead, mercury and other heavy metals, chromium is an important environmental pollutant, mainly concentrated in areas of intense anthropogenic pressure. The effect of potassium dichromate on *Lemna minor* populations was tested using the growth inhibition test. Cyto-histological and physiological analyses were also conducted to aid in understanding the strategies used by plants during exposure to chromium. Treatment with potassium dichromate caused a reduction in growth rate and frond size in all treated plants and especially at the highest concentrations. At these concentrations the photosynthetic pathway was also altered as shown by the decrease of maximum quantum yield of photosystem II and the chlorophyll b content and by the chloroplast ultrastructural modifications. Starch storage was also investigated by microscopic observations. It was the highest at the high concentrations of the pollutant. The data suggested a correlation between starch storage and reduced growth; there was greater inhibition of plant growth than inhibition of photosynthesis, resulting in a surplus of carbohydrates that may be stored as starch. The investigation helps to understand the mechanism related to heavy metal tolerance of *Lemna minor* and supplies information about the behaviour of this species widely used as a biomarker.

The influence of nitrogen and phosphorous status on glyphosate hormesis in *Lemna minor* and *Hordeum vulgare*

Cedergreen, N; Hansen, NKK; Arentoft, BW

European Journal of Agronomy 73: 107-117 (2016)

The herbicide glyphosate has been shown to stimulate growth and photosynthetic capacity of barley and other plant species. The growth increase, however, only takes place under certain, yet undefined, growth conditions. We hypothesise that glyphosate growth stimulation only takes place, when growth is nutrient limited. Nutrient limitation was induced in this study by limiting nitrogen and phosphorous availability. The experiments were performed on hydroponically grown lesser duckweed and barley and on barley in the field. Hydroponic duckweed and barley grown under a range of N- and P-availabilities displayed glyphosate induced growth increases in plants which were slightly stressed by N-deficiency, but not in response to P-deficiency in the case of barley. The growth increase found for P-deficient duckweed was hypothesised to be caused by glyphosate derived P, acting as a nutrient source. No growth increase was found in the 2012 field experiment, which was in contrast to earlier year's findings. Our hypothesis that nutrient limitation makes plants susceptible to glyphosate induced growth was only confirmed for nitrogen but not for phosphorous and not under field conditions in 2012. Mechanisms related to high C:N ratios might be of importance, as this trait varies depending on N- and P-availability during plant growth.

Effect of smoke-derived extracts on *Spirodela polyrhiza*, an aquatic plant grown in nutrient-rich and -depleted conditions

Stirk, WA; Kulkarni, MG; van Staden, J

Aquatic Botany 129: 31-34 (2016)

The effect of smoke-derived extracts (crude smoke-water and karrikinolide [KAR(1)]) on the growth of an aquatic plant *Spirodela polyrhiza* was investigated. Smoke-derived compounds enhanced the growth of *S. polyrhiza*. The effects were also modulated by nutrient concentration with plants growing in nutrient-rich conditions (50% Hoagland's solution) being less sensitive to high concentrations of smoke-water compared to those growing in nutrient-depleted conditions (1% Hoagland's solution). The chemical properties of KAR(1) and its ability to move through both the aerial and soil environments suggest that it is likely to eventually enter the water system. As the present results show that growth of the aquatic *S. polyrhiza* is significantly influenced by smoke-derived KAR(1) with the response modulated by nutrient concentrations, the ecological implications of smoke-derived compounds entering freshwater systems requires further investigation.

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: 189-193 (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 1 mg L-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.

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

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

Aquatic Toxicology 169: 188-195 (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 25 mu g/L) of cylindrospermopsin. Responses with longer exposure periods (48, 96, 168 h) were tested only with exposure to 2.5 and 25 mu g/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 24 h even at the lowest cylindrospermopsin exposure concentrations. Catalase showed the most representative antioxidant response observed after 24 h and maintained its activity throughout the experiment. Catalase activity corresponded with the contents of hydrogen peroxide at 2.5 and 25 mu g/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 (168 h). The results indicate that cylindrospermopsin promotes oxidative stress in *L. minor* at concentrations of 2.5 and 25 mu g/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.

Taxonomy & systematics

Taxonomy of duckweeds (Lemnaceae), potential new crop plants

Sree, KS; Bog, M; Appenroth, KJ

Emirates Journal of Food and Agriculture 28: 291-302 (2016)

Duckweeds are increasingly gaining interest because of their potential as a new aquaculture crop. In the present era of high throughput research, duckweed taxonomy has to be emphasized in order to support and strengthen scientific communication and commercial application. Since the publication of the fundamental monograph on Lemnaceae by E. Landolt in 1986, a number of changes have taken place in terms of their taxonomic position and nomenclature, which we summarize in this review. We report here about the systematic position of this plant family and the changes in its organization. Three additional species were identified; one new genus and subsequently one of the species was re-defined after the publication of the key of determination in 1986. At-present Lemnaceae comprises of 37 species grouped into five genera. We envisage that this review will serve as a compilation of all these recent revisions, describing the state of art of duckweed systematics.

Morphological study of *Lemna minuta Kunth*, an alien species often mistaken for the native *L. minor L.* (Araceae)

Ceschin, S; Leacche, I; Pascucci, S; Abati, S

Aquatic Botany 131: 51-56 (2016)

Lemna minuta Kunth and *Lemna minor L.* are two small aquatic floating plants easily mistakable for similar morphology. The need to distinguish with certainty these two species is a relevant issue, not only from a floristic viewpoint, but also for establishing management plans in wetlands where *L minuta* is an invasive alien species as in Europe. The vein number and frond length are considered by most authors as the main morphological characters discriminating the two species. However, in this study the use of these two characters has not been effective for the determination of 43 specimens out of 248 living specimens of *Lemna* collected from 15 different wetlands of the Central Italy. Statistical analyses of the dataset made it possible to better define the variability of the morphological characters of these two species and to identify additional diagnostic characters to use for improving the morphological discrimination between them. Among these characters, width, apex and shape of the frond, were the most helpful. Decision Trees were elaborated for differentiating *L. minor* from *L. minuta* with greater efficiency both in the laboratory and in the field. The increase in percentage of correct determination of *Lemna* specimens through measurement and parallel utilization of the emerged morphological characters will facilitate the botanists' activities, but also will have practical implications, such as the ability to contribute better to the decision-making system in drawing up plans for plant biodiversity protection and/or eradication of the alien species.

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.

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