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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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This issue is dedicated to

Prof. Satish C. Maheshwari

Felicitation of Prof. S.C. Maheshwari with

Lifetime Achievement Award

at the 4th ICDRA in Kerala, October-2017

Cover page

Prof. Satish C. Maheshwari was felicitated with Lifetime Achievement Award at the 4th International Conference on Duckweed Research and Applications honoring his contributions to the field of Plant Biology in general and Duckweed Biology in specific. Prof. Maheshwari, sadly, passed away on June 12, 2019 in Jaipur, India. As a tribute to his scientific achievements, we dedicate this issue 26 of the 'Duckweed Forum' to Prof. S.C. Maheshwari.

In this issue

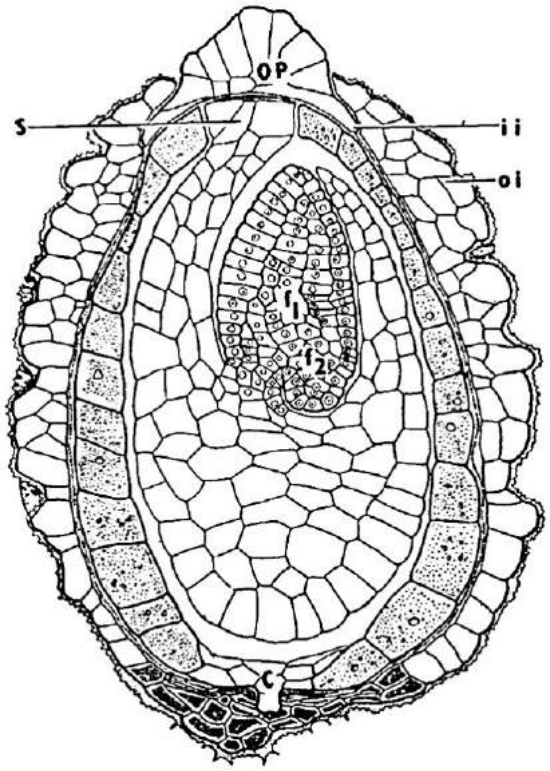
The 3rd International Steering Committee on Duckweed Research and Applications Members

- **Chair: Prof. Eric Lam**, Rutgers, The state University of NJ, New Brunswick, USA; ericL89@hotmail.com
- **PD Dr. Klaus-J. Appenroth**, Friedrich Schiller University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Prof. Marvin Edelman**, Weizmann Institute of Science, Rehovot, Israel; marvin.edelman@weizmann.ac.il
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Padanakkad, India; ksowsree9@cukerala.ac.in
- **Dr. Yubin Ma**, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China; mayb@qibebt.ac.cn
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

- **External Advisor: Tamra Fakhoorian**, International Lemna Association, Mayfield, KY, USA; tamraf9@gmail.com

All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: Endosperm and Seed of Wolffia microscopica (Griff.) Kurz



This drawing is not only a testament to the artistic skills of Late Prof. S.C. Maheshwari but also to his scientific temperament to deal with such minute subjects with great precision. The fact that even after more than 60 years of this work by Late Prof. S.C. Maheshwari we have not yet reached too far in the seed biology of duckweeds, shows how far reached his scientific thought process was.

Figure: "Longitudinal section of mature seed; note the first frond (f_1) and its daughter frond (f_2) (xc. 166). c- caecum, f_1- first frond, f_2- daughter frond, ii- inner integument, oi- outer integument, op- operculum, s- suspensor." (Maheshwari S.C., Nature, 1956). Drawing by Late Prof. Satish C. Maheshwari, Jaipur, India.

Letter from the Editor

Dear Duckweed Community,

On behalf of the International Steering Committee on Duckweed Research and Applications (ISCDRA), I like to send you our greetings with this 26th issue of the Duckweed Forum (DF26), our community's newsletter. As you may notice from our Cover of this issue, we are dedicating this installment to the life and achievements of Prof. Satish C. Maheshwari, affectionately referred to as SCM by those who knew him. A fine Tribute to SCM was contributed by Prof. Sopory and colleagues from India, and it nicely illustrates the history and character of a model scientist who lived a full life pursuing and sharing knowledge. As an example of his remarkable artistic as well as scientific abilities, this issue's Science meets Art section features an illustration of a duckweed embryo from one of the smallest species of the Lemnaceae, *Wolffia microscopica*, published in *Nature* back in 1956 - before many of us were born. To the end, SCM remains committed to the duckweed community and made the effort of attending our last international conference at Kerala in 2017. I still remember his remarkable strength and determination to deliver his Plenary Lecture in spite of the fact that he was unwell at the time, and interacted gracefully with all the students and foreign guests alike. Even in this issue of DF26, he is a contributing author to the historical account of another past giant in the duckweed field - C. F. Hegelmaier. His dedication to our field is truly an inspiration and I am fortunate to have met him in person during our Kerala conference.

Looking toward the present and future for the duckweed community, I have the pleasure to bring you several examples of diverse development. There is a description of an official Training Programme in India to orchestrate the utilization of duckweed, among other potential feedstocks, as replacement for fishmeal in aquaculture, while a start-up in Peru endeavor to achieve similar objectives from a commercial perspective. In parallel, the interest of using duckweed for human consumption is showcased by a recipe book project by high school students from Germany with many enticing photos of healthy salads that incorporate *Wolffia arrhiza* as a common ingredient. Incidentally, our Student Spotlight section also features an account by a student from China who envisions duckweed as part of our solution to food security for this planet. This common thread among these contributions of our Newsletter indicates that there is an international interest in the application of duckweed for human nutrition, whether as feed or food. This clearly should be a topic to watch as our community evolves in the coming year.

In preparation for the upcoming International Conference at Rehovot, Israel, in September 2019, a final "brochure" for the meeting is provided by the organizing committee. It is noted that the registration and submission deadlines have both been extended to early August. Hopefully this will enable more potential participants to attend. I also would like to point out two related items in this Newsletter: First, the election for the next members of the ISCDRA is now open for ballots. The procedure is described in this issue of DF and I encourage everyone eligible for voting to spend a few minutes to cast their ballots before the deadline. We need your participation to ensure that our community will be properly represented. Second, there are two proposals to host our next International Conference in 2021. Please take a moment to read their applications and consider your choice. We will decide the winner for this bid at the meeting in Israel this September. Your educated consideration of these interesting candidates before that event will be most appreciated.

Finally, as in the past, our ISCDRA member Klaus Appenroth has contributed another well-curated Database of publications in the Duckweed arena. I hope you will continue to find this section invaluable for keeping up with the literature and knowledge growth in our field and community.

Best wishes to all, and I hope to meet many of you in Rehovot soon.

Eric Lam, Chair of the ISCDRA

5th ICDRA: Brochure

For further information & registration: www.weizmann.ac.il/conferences/DRA2019

Professor Satish C. Maheshwari (1933-2019)

One of the renowned plant physiologist and molecular biologist of India, Prof. Satish C. Maheshwari (we all called him SCM) passed away on June 12, 2019. He was born on October 4, 1933 in Jaipur. He is survived by his students, well wishers and family members. His wife, Nirmala Maheshwari, herself a plant researcher, scholar and a teacher, passed away a few years back.

Following his early education in Jaipur and Dhaka, SCM moved to Delhi, along with his father, Prof. Panchanan Maheshwari, FRS, who was appointed Professor and Head of the newly created department of Botany at the University of Delhi in 1949. After studying at St. Stephen's college, he finished his studies in Botany and later completed his Ph.D. under Prof. B.M. Johri at the University of Delhi on the embryology of duckweeds. His studies established that with regard to key embryological features, the family Lemnaceae was closely related to Araceae. This work of his was published in *Nature*.

SCM's interest in Lemnaceae continued for many years. He studied the mechanism of flowering in some of the members of this family, which were largely found to be short day plants. The work on the photoperiodic behaviour of *Wolffia microscopica* was also published in *Nature*. However, using cytokinins, along with a chelating agent EDDHA, his group was able to induce flowering even under long day conditions in these duckweeds. One of us (JPK) and another student, Bhaju Tamot (from Nepal) did extensive work on flowering in *Lemna paucicostata* (presently accepted nomenclature: *Lemna aequinoctialis*), *Spirodela polyrhiza, Wolffia microscopica* and found that compounds like salicylic acid, tannic acid, catecholamines, etc. could also induce flowering in some of these species under non-inductive conditions. Interestingly, one of the recent publications of SCM was also on duckweeds, "The duckweed *Wolffia microscopica*: a unique aquatic monocot". To honour the significant contributions SCM made in the field of duckweeds, the 4th International Conference on Duckweed Research and Applications, held in Kerala, was dedicated to him. His participation, keynote lecture and interaction with a number of other participants from across the world will surely be remembered for a long time to come.

Another group of students were engaged in isolating and characterizing cytokinin from seeds of watermelon. When one of us (SKS) joined his lab, we were asked to characterize cytokinin in tRNAs, a work that could not be followed for some reasons and SKS ended up continuing work on anther culture for the production of haploids, a landmark discovery that was made by Guha and Maheshwari in mid 1960's and had been published in a few papers in *Nature*. Impact of this

discovery was noticed as a number of labs across the globe took on this method for making haploids and dihaploids. This discovery from SCM's lab was also cited by JD Watson in his book.

SCM was very keen to understand the biochemical and molecular mechanisms underlying cell differentiation and development. Following his visits abroad at different time points to Yale University, California Institute of Technology, Oxford University and Harvard University, where he learnt and undertook research on RNA synthesis and presence of DNA in organelles, he established the Unit for Plant Cell and Molecular Biology, at the Botany Department which later on evolved into a full fledged Department of Plant Molecular Biology at the University of Delhi, South Campus; one of the first of its kind in the Country. During these years, almost spanning from mid 1970's to his superannuation in 1995 and later, he started work on photobiology, especially on characterization of phytochrome in wheat, on early events triggered by the active form of phytochrome, including phosphorylation of organelle proteins. These studies induced many of his students and others in the country to enter into the field of plant molecular biology. It wouldn't be out of place to mention that SCM can be credited with ushering in plant molecular biology work by asking the Ministry of Science and Technology, Government of India, to start Centres of Plant Molecular Biology in the country in the late 1980s. In fact, he was the first in the country to get work started on *Arabidopsis* in his lab during the early 1960s; a plant which has since been used as the most versatile plant model system. With the establishment of the new Department, SCM also embarked on investigations involving recombinant DNA and gene regulation. With one of us (AKT), he established mode of interaction between light and development-dependent signals leading to control of mRNA levels of chloroplast-encoded genes in rice. Extension of this work unravelled role of phytochrome, calcium and phosphorylation in light-dependent expression of plastid genes.

After his retirement from the University of Delhi, he joined the International Centre for Genetic Engineering and Biotechnology (ICGEB) as a Visiting Scientist, where one of us (SKS) was working. Together we organized an International meeting on Signal Transduction, a favourite topic of SCM, which was published as a volume by Plenum Press. Together we also edited a special section on plant molecular biology for the journal *Current Science*, in which persons like A.W. Galston, L. Bogorad, with whom he had worked earlier, sent their contributions. Being very keen on knowing the history of development for a subject, we also wrote an article on the early days of modern plant biology work in India. During his later tenure at ICGEB, he was very keen that some group should take up work on apomixis.

After the death of his wife, Nirmala Maheshwari, SCM shifted to Jaipur where he continued to stay in touch with literature, prepare his lectures, which he used to do very methodically. The scientific community will surely miss listening to his lectures. During his stay in Jaipur, SCM was regularly visiting the University of Rajasthan and was keen to interact with the young students and share his knowledge and expertise with them, inspiring them to develop scientific temper. Although traveling long distances in flights was not an option during his later years, SCM was acquainted well enough with the technological advancements to travel to different labs across the globe via internet and to update himself with the most recent happenings in the field of plant biology and molecular biology in general.

During his academic career, SCM received many honours and awards. To mention a few: he received the Shanti Swarup Bhatnagar prize from the Council of Scientific and Industrial Research, India; Goyal Prize, J.C. Bose Medal, Birbal Sahni Gold Medal of the Indian Botanical Society, which also elected him as its President; J.J. Chenoy Award from the Indian Society of Plant Physiology, Homi Bhabha Fellowship and Jawaharlal Nehru Fellowship; and D.Sc. (Honoris Causa) from the University

of Hyderabad. He was also elected Fellow of the Indian National Science Academy, the Indian Academy of Sciences and the National Academy of Sciences, India.

SCM's life has basically revolved around books, journals, writing, and lectures. He loved to educate himself and also others. Besides academics he had interest in western and Indian classical music.

SCM was a very demanding supervisor and tough administrator but internally he was very soft, affectionate and helpful at the same time. He lived his life on his own terms till the last moment. SCM will be missed by his students, colleagues and friends alike.

Sudhir K. Sopory[1], Jitendra P. Khurana[2], Akhilesh K. Tyagi[2], K. Sowjanya Sree[3]

[1]International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India

[2]Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India

[3]Department of Environmental Science, Central University of Kerala, Periye, India

Historical account: Christoph Friedrich Hegelmaier (1833-1906)

The then expert on Lemnaceae

K. Sowjanya Sree[1], Satish C. Maheshwari[2†], Klaus-J. Appenroth[3]
[1]Dept. Of Environmental Science, Central University of Kerala, India; [2]Centre for Converging Technologies, University of Rajasthan, Jaipur, India; [3]Matthias Schleiden Institute- Plant Physiology, Friedrich Schiller University of Jena, Germany; [†]Passed away on June 12, 2019

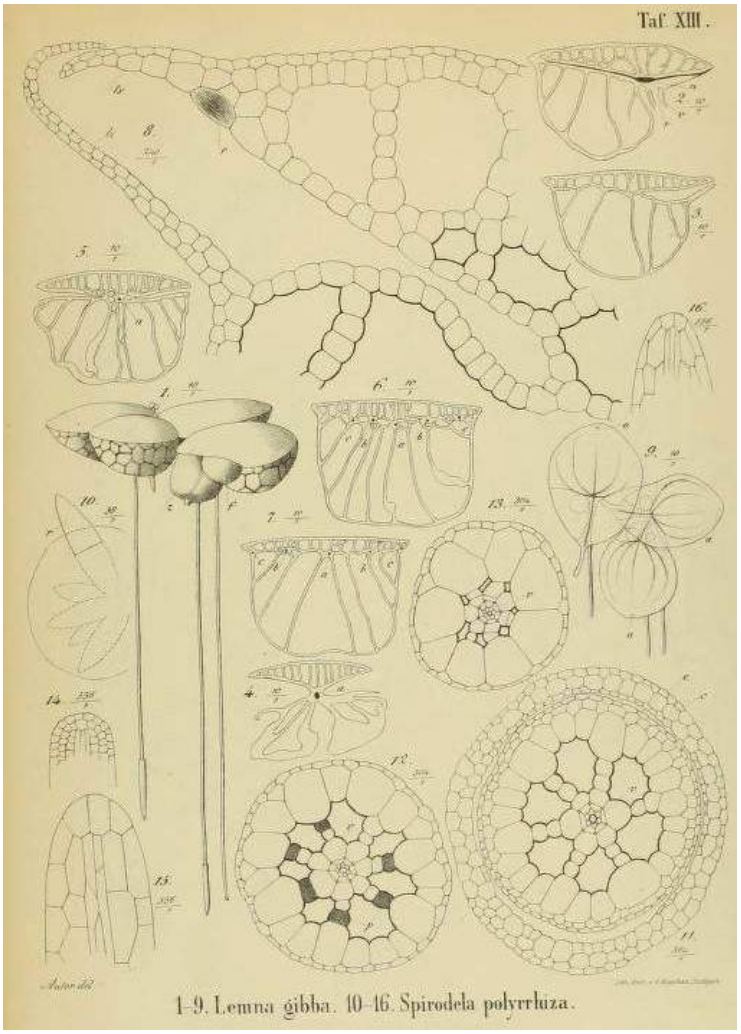
Indo-German scientific interactions and collaborations in the field of duckweed research have not only been active in the current decades but have been of interest in the past few centuries as well. Going back in time for more than a century and discussing about duckweed research, one will surely come across the then authority on the duckweed plant family, Christoph Friedrich Hegelmaier (1833 - 1906). Even without a keen historical interest, duckweed researchers and botanists must be familiar with the name of C.F. Hegelmaier. His name is connected with quite a number of duckweed species as an author (Hegelm.) who discovered and/or classified them taxonomically: *Lemna disperma* Hegelm., *Wolffiella denticulata* (Hegelm.) Hegelm., *Wolffiella gladiata* (Hegelm.) Hegelm., *Wolffiella lingulata* (Hegelm.) Hegelm., *Wolffiella oblonga* (Phil.) Hegelm., *Wolffiella welwitschii* (Hegelm.) Monod, *Wolffiella repanda* (Hegelm.) Monod, *Wolffia cylindracea* Hegelm. (Sree et al., 2016).

C.F. Hegelmaier was born in Sulzbach on 4 September 1833, presently placed in the state of Baden-Wuerttemberg, Germany, not far from Tuebingen. He was primarily a physician from his study in medicine at University of Tubingen, Germany. However, he was also very much interested in Botany. After he earned his PhD in 1857 from University of Tuebingen and habilitated from the same University in 1864, he joined the same University as an Associate Professor of Botany (Mägdefrau, 1969). He worked together with the well-known scientists Simon Schwendener (The German Botanical Society's highest medal is in his honor; and it is pertinent to mention here that one of us, KJA, is a recipient of this medal) and Wilhelm Pfeffer (In his honor, the Wilhelm Pfeffer foundation supports talented young scientists).

Hegelmaier mainly investigated the anatomy and morphology of the members of duckweed family though his merit is that, he also studied their developmental history. It is worth mentioning that his investigations on embryology were, for a long time, the basis of general embryology of monocots and dicots. Also, function and development of the endosperm were part of his investigations. Not only that his scientific studies as an expert had long term impact, but his student project on experimental psychology was used in psychophysics for more than a century (Laming and Laming, 1992).The monograph "Die Lemnaceen" published in 1868 is, till today, a rich source of knowledge on Lemnaceae. It has more than 10 editions, the last one being in 2010. Apart from the morphology and anatomy of the fronds, the monograph also details flowering, seed morphology, germination, development and embryology, development of roots and systematics of different members of

Lemnaceae. Apart from the text, the drawings play a significant role in attracting the readers to this monograph, till date. Moreover, the publication in 1895 is a kind of summary of his life-long work with duckweeds concerning their systematics. Besides duckweed he also investigated *Callitriche*, a genus within the plant family, Plantaginaceae.

Hegelmaier investigated each problem very carefully. He always mentioned possible arguments against his results and remained very critical even towards his own conclusions, making the language of his writings often complicated. All his publications were in German, however, even the native speakers find it difficult to translate them. One very praiseworthy attitude was that Hegelmaier was ready to contradict conclusions drawn by himself, if necessary, and to correct them based on further and newer investigations and results thus obtained and by providing a detailed justification. One such example was with his studies on a duckweed member, *Wolffia microscopica*. Based on the available literature, Hegelmaier in 1868 classified *W. microscopica* as a stipitate duckweed species (a species where the stipe remains with the daughter upon abscission from the mother frond). However, later upon investigating a sample received from his colleague from the then India, he realized that the structure that he considered as an overgrowth of the stipe was actually a root-like structure which he later called as rhizoid. Hegelmaier then published these results and further in an exclusive publication on this species in 1885, correcting the conclusions drawn by himself previously.



A drawing plate from the monograph "Die Lemnaceen"

Although Hegelmaier left this world on 26 May, 1906, his herbarium still exists as a valuable resource and is available at the Museum of Natural History in Stuttgart “Staatliches Museum für Naturkunde Stuttgart”, also in the state Baden- Wuerttemberg, Germany.

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The authors (from left to right): Late Prof. Satish C. Maheshwari, Dr. K. Sowjanya Sree, Dr. Klaus-J. Appenroth.

Report on a Training Programme

on Recent Advances in Fish Nutrition with special focus on duckweed as a source

R. Chakrabarti[1] and J. G. Sharma[2]

[1]Aqua Research Lab, Department of Zoology, University of Delhi, Delhi 110 007, Email: aquaresearchlab@yahoo.co.in; [2]Department of Biotechnology, Delhi Technological University, Bawana Road, Delhi 110 042, Email: sharmajaigopal@yahoo.com

With changing scenario in world agriculture, attempts are being made to maximize the fish production per unit area. Hence, it has been imperative to provide quality feed regularly to fish cultured in inland water bodies. The commercially available feeds are usually cost-prohibitive and their sole objective is to increase only fish growth. In February 2015, Department of Biotechnology-Biotechnology and Biological Sciences Research Council (DBT-BBSRC) took an initiative to formulate a research programme with the objective to develop a low cost fish feed with easily available, non-conventional local ingredients in order to increase the nutritional value of the cultured fish. The targeted beneficiaries would be the fish farmers of under developed and developing countries. Thus, the scientists from various Institutes of Africa, India, Bangladesh and UK were invited to participate in the project formulation. There were several rounds of discussions and brain storming sessions. Finally, the project was formulated and started in April 2016. Duckweeds *Lemna aequinoctialis* and *Spirodela polyrhiza* are selected as suitable ingredients for fish feed formulation. These two macrophytes are rich sources of amino acids, polyunsaturated fatty acids, minerals etc. Thus, the feed prepared with these ingredients will improve the nutritive value of the fish produced for human consumption. To disseminate the research findings among the scientific community, a training programme on “Recent Advances in Fish Nutrition” was organized by Aqua Research Lab, Department of Zoology, University of Delhi during March 15-17, 2019.

Release of Training Manual by Prof. Jitendra P. Khurana, Honourable Pro-Vice-Chancellor, University of Delhi.

Twenty-five candidates from four different countries - Kenya, Tanzania, Bangladesh and India were selected for this training programme. Indian participants were from nine different states. All selected candidates are actively involved in aquaculture. The programme was inaugurated by the **Honourable Pro-Vice-Chancellor of the University of Delhi, Prof. Jitendra P. Khurana**. He released the Training Manual and also shared his research experiences with

duckweeds, especially molecular aspects. His graceful presence and valuable talk were a great inspiration for the young participants. The three days training programme was organized in such a way that participants would get proper exposure to the advanced tools and techniques in the field of Fish Nutrition along with theoretical background.

On the first day, March 15, there were both lectures and practical sessions. Prof. Rina Chakrabarti in her lecture "Freshwater Macrophytes: Potential Ingredients for Fish Feed Formulation" introduced the participants to the world of duckweeds. She talked about the environmental and nutritional requirements for the large scale production of duckweeds. She also informed the participants about the nutritional value of two duckweed species *Lemna aequinoctialis* and *Spirodela polyrhiza* in terms of amino acid and fatty acid compositions; also their suitability for fish feed formulation. Dr. Girish Mishra, Department of Botany, University of Delhi gave a talk on "Microalgal Feed towards Enhancement of Omega-3 Polyunsaturated Fatty Acids". He had given in depth information about the role of PUFA in fish feed formulation.

On the second day, March 16, there were both lectures and practical sessions. Digestive enzymes play a key role in bioavailability of ingested feed to fish. Prof. Rina Chakrabarti gave a lecture on "Digestive Enzyme Profile of Carps". She used carps as model fish and explained how digestive enzyme profiles varied during ontogenic development. She emphasized on species-specific and age-specific feed formulation for fish. Prof. B. D. Banerjee, Department of Biochemistry, University College of Medical Sciences, Delhi discussed about the importance of amino acids for fish nutrition as well as human nutrition in his talk on "Amino acids: Building Architecture of Protein and Life".

On the third day, March 17, there were also lectures and practical sessions. The programme was started with the talk of Prof. Sunil Pabbi, Division of Microbiology, IARI, New Delhi. He delivered his lecture on "*Spirulina*: A Balanced, Nutritious Supplement for Aquaculture". He discussed about the large scale production of this algae and its nutritional value for fish. Dr. K. Sowjanya Sree, Department of Environmental Science, Central University of Kerala, Kerala discussed about the

"Happy Moments" participants are with Mentors at the end of the three days Training Programme.

potential applications of duckweeds in fish feed formulation in her talk "Nutritional Value of Duckweed". Her speech was full of academic and practical information.

In these three days, participants had learnt techniques related to amino acids, fatty acids, gene expression, *in vitro* digestibility study, water quality analysis etc. Some of these techniques require thorough knowledge of the instruments. Therefore, before the start of the practical exercise, experts in the specific field gave lectures about the instruments. Mr. Ashok Kumar, Lab India talked on "Operation of Hitachi L-8900 - A Researcher Friendly Equipment for Amino Acids Analysis". Mr. Abhay Singh Chandel, Perkin Elmer discussed on "Functional Mechanism of Gas Chromatography" and Mr. Umesh Sharma, Thermo Scientific delivered a talk on "qPCR - Basics and Its Applications". The participants got thorough knowledge of instrumentation and were comfortable in performing their practical under the guidance of members of Aqua Research Lab.

The entire programme was efficiently anchored by Padma Shri Prof. Ravi Chaturvedi. The programme was concluded by distribution of Certificates to the participants through the former Adviser, Department of Biotechnology & Vice-Chancellor of Birsa Agricultural University, Ranchi, Prof. George John. In his remarks, he stated the importance of Fish Nutrition for sustainable Aquaculture.

Jungleponic

A Local Sustainable Aquaculture providing a fish-in feed solution, Iquitos, Peru

Matthew Hay
Jungleponic, Iquitos, Peru

Jungleponic is a sustainable aquaculture start-up developing an application for duckweed as an alternative protein input for feeds.

Based in the city of Iquitos in the Peruvian Amazon, the company's mission is to introduce fully sustainable aquaculture to the region with zero fish-in feed solutions which reduce the environmental impact and cost of aquaculture and animal feeds in the Peruvian Amazon basin.

Although rich in flora and fauna, the region does not produce the proteins required to sustain substantial fish or animal production. It is also difficult to access; with a population of over half a million, Iquitos can only be reached by river or air. Its isolation, tropical climate, and freshwater resources create an opportunity for locally-produced, alternative proteins to replace imported soy and fishmeal. This is an ideal application for duckweed.

The first objective for Jungleponic is to formulate duckweed-rich diets for predominantly herbivorous, native fish species. These fish are consumed locally or used as live-feed in the production of carnivorous export species.

Spirodela grown in our pond-based production demonstration site

Co-founder, Matthew Hay, collecting native Lemna strains, Tarapoto, Peru

Jungleponic is currently validating pond-based production using organic inputs sourced from the region and treated with enhanced microorganisms. The current production is focused on Lemna and Spirodela. This demonstration project is funded through an award from the Peruvian government for biological innovation. The initial results are promising, and the company has been shortlisted for a national innovation program in fishing and aquaculture.

Jungleponic was founded in 2018 by Matthew Hay, Rossy Hernandez, and Braulio Noriega. The team has entered into joint venture with Lima-based consultancy Quantum Analysis, led by Energy and Agriculture specialist Dante LaGatta. The goal is to develop an integrated project (proteins, feeds, processing, co-generation and refrigeration) based on the circular-economy model, which further supports the company's mission to create lasting environmental, economic, and social impact.

We are looking for stakeholders interested in our endeavor to produce commercial duckweed animal feeds and processing high-grade Amazon fish i.e. Paiche, ornamentals and others.

Tiny Plants – Huge Opportunities

The potential of spotless watermeal (*Wolffia arrhiza*) for human nutrition

Klaus-J. Appenroth

Matthias Schleiden Institute- Plant Physiology, Friedrich Schiller University of Jena, Jena, Germany

Several students from the Government Secondary School in Neustadt/Orla, Germany studying from school classes 11 and 12 found it interesting to read about the benefits of adopting the duckweed *Wolffia* for human nutrition. Taking this interest further, Patricia Berthold, Nathalie Fritz, Paula Jauch and Lilly Timm (see photo below) had cultivated *Wolffia arrhiza* (strain 9528 from Germany obtained from the Duckweed Stock Collection of Klaus-J. Appenroth, Friedrich Schiller University of Jena, Germany) as the European equivalent of the tropical species *Wolffia globosa* and used the harvest to make quite a lot of sophisticated and delicious meals. In order to share their excitement and experience with others, they had now formulated a book of recipes. Reading their recipe book in this newsletter, one can feel the joy that these young ladies must have experienced while working with *Wolffia* for their thesis, which they had submitted before their final school examination after class 12 (Matura). They have also submitted the thesis to government level competitions in East Thuringia and the state Thuringia of Germany and were honored with leading awards. This seminar paper was supervised by their school teacher, Virginia Mudrich and by the external supervisor, Klaus-J. Appenroth.

Savory recipes around the spotless watermeal

Fast and healthy for everyday life

Great recipes around the spotless watermeal for every occasion and every taste!

Due to the neutral taste of the spotless watermeal it can be quite versatile in the kitchen. There are a lot of possibilities to integrate the plant into dishes.

In this recipe book, the variety ranges from light salads to spicy spreads to tasty recipes for main dishes with the little green plants.

Good luck in trying out and enjoying!

Savory recipes around the spotless watermeal

Fast and healthy for everyday life

Did you know that the spotless watermeal ...

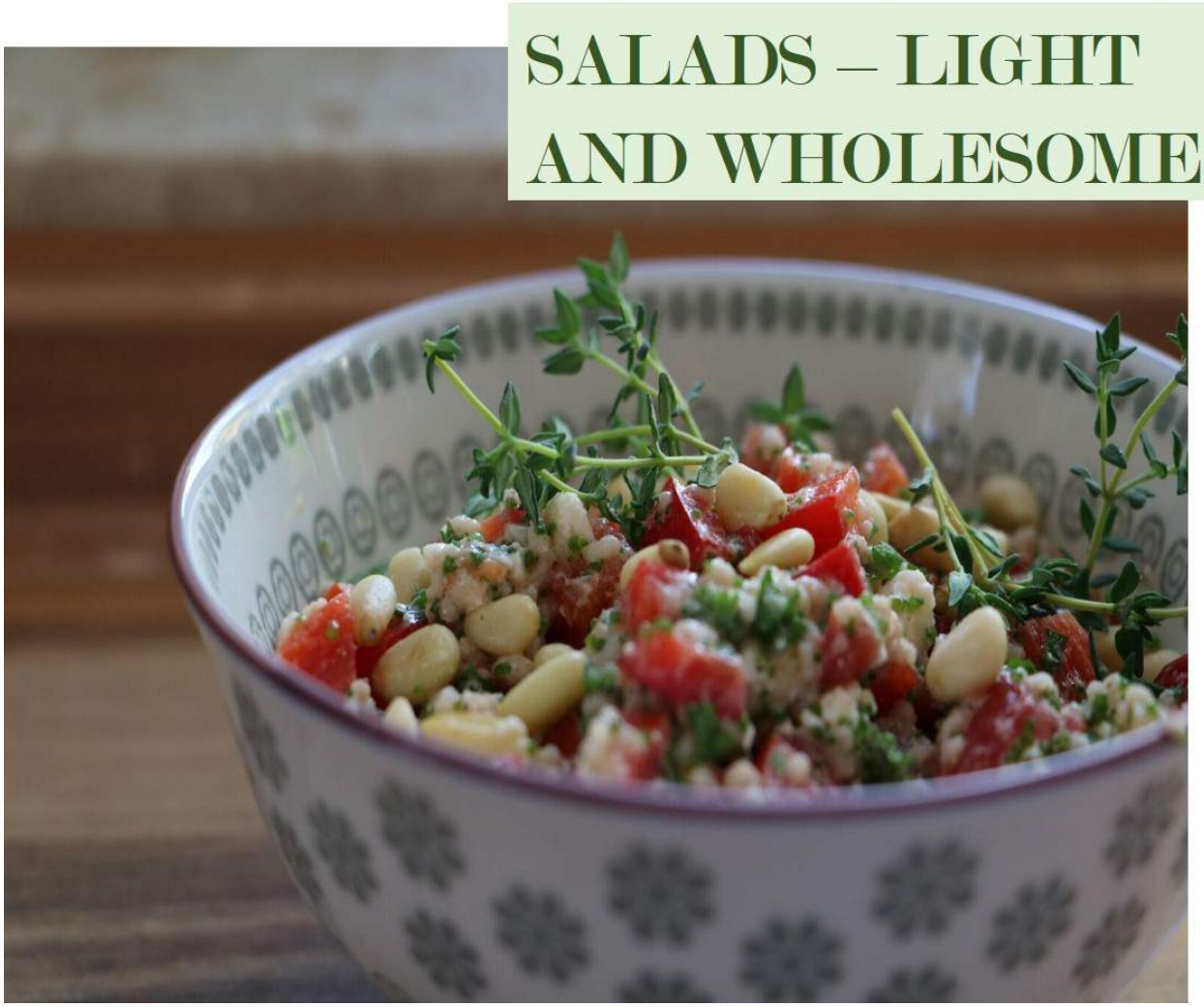
... can have varied use in sweet-fruity as well as hearty dishes because of its neutral taste?

... is already in a lot of dishes in Asian countries?

... has very healthy and high quality proteins and fats?

... is the smallest flowering plant in the world and can double its mass after just a few days?

Spotless watermeal omelette


SALADS – LIGHT
AND WHOLESOME

Mango salad with spotless watermeal

Ingredients

1 mango; 1/2 pomegranate; 2 tbsp. spotless watermeal; 3-4 mint leaves; 2 stems leaf parsley; 2 tsp. lemon juice; chili flakes

Preparation time 10 minutes

Preparation

1. peel mango and cut it into bite-sized pieces
2. cut open pomegranate, remove the seeds with a teaspoon and add them to the mango pieces
3. add spotless watermeal to the mango and pomegranate
4. add lemon juice
5. roughly chop the parsley and mint and add to the mix
6. mix all ingredients and season with chili flakes as desired

For 2 portions

Couscous salad with spotless watermeal

Ingredients

100g couscous; 3 tbsp. spotless ; watermeal; 1/2 clove of garlic; 1 pepper; 2 medium-sized tomatoes; 75g sheep's cheese; 2 tbsp. of olive oil; 2 stems of each thyme & parsley; 25g pine nuts; salt & pepper

Preparation time 15-20 minutes

Preparation

1. let the couscous swell up in hot water for 10 minutes and then drain the excess water
2. wash and cut the pepper and tomatoes
3. finely chop the garlic and herbs and add to the couscous and vegetables
4. add spotless watermeal
5. cut the sheep cheese and mix with oil
6. roast pine nuts in a pan for 5 minutes
7. mix pine nuts in the salad and season with salt and pepper as desired

For 3 portions

SPREADS – FAST AND PIQUANT

Herbal spotless watermeal spread

Ingredients

125g double cream cheese; 100g grainy cream cheese; 2-3 tbsp. spotless watermeal; 1 knife tip chopped chili pepper; 5 stems of leaf parsley; 1/2 clove of garlic; curry & ground ginger; salt & pepper

Preparation time 10 minutes

Preparation

1. mix the double cream cheese and the grainy cream cheese
2. add coarsely chopped parsley
3. add finely chopped garlic
4. mix in spotless watermeal
5. finely chop the top of a chili pepper and add to the mixture
6. season with salt, pepper, ginger and curry as desired

For 5 people

TIP

The spread tastes best on wholemeal bread or crispy crispbread

Spotless watermeal guacamole

Ingredients

1 avocado; 1 tbsp. double cream cheese; a tip of finely chopped chilli pepper; 1 clove of garlic; 1 tbsp. spotless watermeal; 2 tbsp. lemon juice; 2 stems leaf parsley; salt & pepper as well as ginger and chilli powder; pine nuts as garnish

Preparation time 15 minutes

Preparation

1. cut avocado in half, remove core and hollow out
2. crush lemon juice and avocado with a fork until creamy
3. add the cream cheese
4. finely chop the tip of the chilli pepper & garlic clove and mix with the mixture
5. coarsely chop and add leaf parsley
6. mix in spotless watermeal
7. season the spread to taste with pepper, salt, ginger and chilli powder
8. sprinkle pine nuts over the guacamole for the garnish

For 4 people

MAIN DISHES - FAST AND WHOLESOME

Indian Naan bread with curry dwarf watermeal topping

Ingredients

2 indian naan breads; 150g double cream cheese; 2 tsp. ginger powder; 2 tsp. curry powder; 2 stem thyme; 2 tbsp. spotless watermeal; salt, pepper & chilli flakes; 20g mongoose beans; 1 fig

Preparation time 15 minutes

Preparation

1. mix cream cheese with ginger powder and curry
2. coarsely chop parsley and thyme and add to the mixture
3. mix in the spotless watermeal
4. season the mixture with salt, pepper and chilli flakes and spread on the naan breads
5. cut the fig into slices and use them together with the mongoose beans as garnish

For 2 portions

TIP

Naan bread can be bought in health food shops or organic markets. But you can also bake it yourself.

Spotless watermeal omelette

Ingrediens

3 eggs; 200 ml milk; 2 tbsp. spotless watermeal; 2 stems leaf parsley; salt & pepper; some oil

Preparation time 10-15 minutes

Preparation

1. beat the eggs and mix with milk
2. coarsely chop the leaf parsley and mix with the egg mass
3. add spotless watermeal
4. season with salt and pepper
5. heat the pan with oil
6. add the egg mass and let it slowly coagulate at low heat
7. lift the omelette out of the pan after 5-10 minutes
8. add more salt and pepper if needed

For one portion

IMPRINT: This recipe book was created as part of a German seminar paper on the topic “Tiny plants-huge opportunities: The potential of the spotless watermeal (*Wolffia arrhiza*) as human nutrition” by Patricia Berthold, Nathalie Fritz, Paula Jauch and Lilly Timm in 2018. All used pictures are from own source. (pictures: Patricia Berthold). Abbreviations: tsp. Teaspoon; tbsp. Tablespoon.

Student Spotlight: Changjiang Yu

Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, China
(Email: yucj@qibebt.ac.cn)

Food security has always attracted numerous concerns by humans for thousands of years due to technical deficiencies in agricultural sciences. Though farmers have been carefully selecting crops with higher yields and with resistance to disease and pests, more efforts still need to be devoted. On the contrary, the human population has surged since the recent two or three hundred years. In less than a decade after first commercialization, international adoption and diffusion of biotech crops has now gone global, especially in developing countries such as my native land, China. From my point of view, plant biotechnology is the key to a better life by not only providing nutritionally enhanced foods, but also by stabilizing the Genuine Progress Indicator and preventing economic crisis with Genetically Modified crops which are available for the poor. Unfortunately, rise in the price of crops early this year predicted potential economic crisis to follow.

Changjiang Yu attending the 3rd ICDRA in Kyoto, Japan

Duckweed, the fastest growing angiosperms, are increasingly gaining attention as potential crop plants due to its high starch and protein content. In 2012, after I got my Master's degree from China Agricultural University, I was fortunate to be given the opportunity to join Drs. Gongke Zhou and Yubin Ma's laboratory. When I first met duckweed, I was very interested in how duckweed can grow so fast and how they accumulated starch to such high content. It seems that understanding the starch accumulation mechanism of duckweed will help human survival by providing healthy and safe food. Therefore, I chose to study the starch accumulation mechanism and applications of duckweed as my PhD project and as a long-term direction.

During my PhD studies, I was working on large-scale screening of duckweeds of different phenotypes, using high-throughput sequencing technology to screen the most important regulation factors in the starch accumulation pathway, and employing transgenic technology to verify their functions afterwards. I have already identified some candidate genes in duckweed and hope they will be useful for food improvement. In May 2019, I passed my PhD defense. Now I am working as an associate professor in Gongke Zhou and Yubin Ma's Lab in Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, China. We are working on duckweed-based

wastewater treatment system, molecular mechanism of starch accumulation in duckweed, duckweed genome analysis and duckweed-based synthetic biology systems.

I appreciate the privilege to attend the 3rd ICDRA in Kyoto in 2015. It was a great chance for me to learn about exciting fields of duckweed research, and to talk with the researchers making these progress.

In 2018, I got the chance to go to University of Georgia, USA to work with Prof. Debra Mohnen (Complex Carbohydrate Research Center, University of Georgia, USA). I had learned lots of new techniques such as sugar analysis and received plenty of helpful comments and suggestions for my study.

Duckweed-based wastewater system at Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, China

Duckweed are remarkable plants with huge potential for economic benefits. However, there are still lots of issues unknown. I hope to be able to start intense collaboration with other researchers in this field for better solutions of food security.

ISCDRA 2019 elections: now open

The elections for the 4th International Steering Committee on Duckweed Research and Applications 2019 are now open. The e-ballot is available at https://tinyurl.com/iscdra2019voting .

Please note that voting will be open until August 31, 2019.

The five newly elected members will elect the Chair of the Committee. In case all the elected members are either from Research or from Applications background, the newly elected Chair will appoint one additional member from the missing field. The duty will be transferred to the newly elected ISCDRA during the general assembly of the ISCDRA scheduled as part of the 5th ICDRA to be held at Weizmann Institute of Science, Rehovot, Israel in September, 2019.

You must belong to at least one of the categories listed below to be eligible to vote.

- Attended any of the past two ICDRA meetings.
- Will attend ICDRA 2019 in Israel.
- A principle investigator who is working with duckweed in his/her laboratory.
- A researcher/postdoc/student who is working with duckweed in their research (i.e. not just reading about it, but actually doing a project).
- An entrepreneur who is working to commercialize a duckweed-based technology and/or product.
- A venture capital principle who has invested significantly into duckweed-based technology and/or product.
- A worker in a commercial venture involved with a duckweed-based technology and/or product.
- A contributing member of the International Lemna Association.

List of candidates for the 4th ISCDRA elections:

- Eric Lam (Rutgers University, New Brunswick, USA)
- Sowjanya Sree K (Central University of Kerala, Periye, India)
- Klaus Appenroth (Friedrich Schiller University of Jena, Germany)
- Tsipi Shoham (GreenOnyx Ltd., Tel Aviv, Israel)
- Yubin Ma (Qingdao Institute of Bioenergy and Bioprocess Technology, Qingdao, China)
- Shuqing Xu (University of Muenster, Germany)
- Robert Martienssen (Cold Spring Harbor Laboratories, New York, USA)

Applications to host ICDRA-2021

The ISCDRA received two applications for the call to host the ICDRA meeting in 2021. We hereby present these two applications to the duckweed community.

Application No. 1: IPK, Gatersleben, Germany

The Institute of Plant Genetics & Crop Plant Research (IPK) in Gatersleben, Germany

invites you to the 6th ICDRA 2021

The IPK is a member of the **Leibniz Association** of 86 non-universitarian German Research Institutions.

(Gottfried Wilhelm Leibniz 1646-1716 "theoria cum praxi„)

IPK at a Glance

Long-standing history in plant research

Host of various international conferences as Gatersleben Research Conferences

One of the largest crop research institutes in Europe

Employees: total ~560; scientists ~180; PhD students ~80 from >30 nations

4 Departments: Genbank & Evolution

Breeding Research (since 2014 cytogenomic research on duckweeds)

Molecular Genetics

Physiology and Cell Biology

IPK as conference venue

Modern lecture hall (225 seats)

Seminar rooms for satellite meetings and parallel sessions; catering from own

Canteen; Accommodation in Quedlinburg (UNESCO world heritage city); bus

transportation organized

Airports: Halle/Leipzig, Hannover, Berlin, ~2 hours by car or train to Gatersleben

Quedlinburg – UNESCO World Heritage City

Welcome 2021 in Gatersleben !

Contact details:

Prof. Dr. Ingo Schubert

IPK, Gatersleben

Germany

Email: schubert@ipk-gatersleben.de

Application No. 2: Guangdong University of Petrochemical Technology, Maoming, China

We are writing this letter as an application to host the ICDRA 2021 in Maoming, Guangdong Province, China. As the host, Guangdong University of Petrochemical Technology (GUPT) is located in Maoming in the southwest of Guangdong Province in China and on the coast of South China Sea. This area has experienced a huge economic growth in the last three decades.

Guangdong University of Petrochemical Technology, Maoming, China

Duckweed research and application has also been growing in the area. We have very active duckweed research projects in School of Environmental Science and Engineering as well as collaborative projects with Hunan University and Peking University-Shenzhen Graduate School and industrial partners. Our duckweed research includes genetic identification of local duckweed, application of duckweed for agricultural and industrial wastewater treatment, and using duckweed for removal of heavy metals and antibiotics from contaminated water bodies. Our recent articles of duckweed systems are published on *Bioresource Technology*, *Journal of Plant Research*, *PLANTA*, *Plant Biology*, *Plant Research*, and *Water Research*.

Maoming city, China

Maoming is quite convenient for travelers. We have a high-speed railway system which connects us to the neighboring cities in Guangdong Province such as Guangzhou, Shenzhen, and Zhanjiang. There are two airports available for the area, Guangzhou Baiyun Airport (CAN) (about 3 hours away by train) and Zhanjiang Airport (ZHA) (about 40 minutes away). There are many tourist resources on the coastal area. http://web3.gdupt.edu.cn/renshichu/index.php/Index/Show/index/id/1.html

Guangdong University of Petrochemical Technology will be responsible for organizing the conference. This application is co-sponsored by our industrial partner, Sumsan Environment Management Company in Shenzhen, which will be responsible for the organization and financial support for a technical tour in Shenzhen to visit the city and a duckweed-based wastewater treatment facility. Shenzhen is the most booming city in China and probably in the whole world in the last 30 years. It is about three and half hours from Maoming by train.

A view of the University auditorium

We hope to have an opportunity to serve the international duckweed community. Once being granted the opportunity, we will form the organizing and program committees for the conference and provide timely reports of the progress to the ISCDRA committee. Please let us know if you have any questions.

Sincerely,

Chunping Yang, Professor and Associate Dean (http://ee.hnu.edu.cn/info/1007/10951.htm)

Jay J. Cheng, Adjunct Professor

School of Environmental Science and Engineering (http://web2.gdupt.edu.cn/hjkxygcxy/)

Guangdong University of Petrochemical Technology (http://www.gdupt.edu.cn/)

Maoming, Guangdong Province

China

Beach at Maoming city, China

From the Database

Highlights

Efficient genetic transformation and CRISPR/Cas9-mediated genome editing in *Lemna aequinoctialis*

Liu, Y; Wang, Y; Xu, S; Tang, X; Zhao, J; Yu, C; He, G; Xu, H; Wang, S; Tang, Y; Fu, C; Ma, Y; Zhou, G (2019) PLANT BIOTECHNOLOGY JOURNAL DOI:10.1111/pbi.13128

The fast growth, ease of metabolic labelling and potential for feedstock and biofuels production make duckweeds not only an attractive model system for understanding plant biology, but also a potential future crop. However, current duckweed research is constrained by the lack of efficient genetic manipulation tools. Here, we report a case study on genome editing in a duckweed species, *Lemna aequinoctialis*, using a fast and efficient transformation and CRISPR/Cas9 tool. By optimizing currently available transformation protocols, we reduced the duration time of *Agrobacterium*-mediated transformation to 5-6 weeks with a success rate of over 94%. Based on the optimized transformation protocol, we generated 15 (14.3% success rate) biallelic LaPDS mutants that showed albino phenotype using a CRISPR/Cas9 system. Investigations on CRISPR/Cas9-mediated mutation spectrum among mutated *L. aequinoctialis* showed that most of mutations were short insertions and deletions. This study presents the first example of CRISPR/Cas9-mediated genome editing in duckweeds, which will open new research avenues in using duckweeds for both basic and applied research.

Low genetic variation is associated with low mutation rate in the giant duckweed

Xu, SQ; Stapley, J; Gablenz, S; Boyer, J; Appenroth, KJ; Sree, KS; Gershenzon, J; Widmer, A; Huber, M (2019) NATURE COMMUNICATIONS 10: 1857

Mutation rate and effective population size (Ne) jointly determine intraspecific genetic diversity, but the role of mutation rate is often ignored. Here we investigate genetic diversity, spontaneous mutation rate and Ne in the giant duckweed (*Spirodela polyrhiza*). Despite its large census population size, whole-genome sequencing of 68 globally sampled individuals reveals extremely low intraspecific genetic diversity. Assessed under natural conditions, the genome-wide spontaneous mutation rate is at least seven times lower than estimates made for other multicellular eukaryotes, whereas Ne is large. These results demonstrate that low genetic diversity can be associated with large-Ne species, where selection can reduce mutation rates to very low levels. This study also highlights that accurate estimates of mutation rate can help to explain seemingly unexpected patterns of genome-wide variation.

Biotechnology

Plant-Made Antibodies: Properties and Therapeutic Applications (REVIEW)

Komarova, TV; Sheshukova, EV; Dorokhov, YL (2019) CURRENT MEDICINAL CHEMISTRY 26: 381-395

A cost-effective plant platform for therapeutic monoclonal antibody production is both flexible and scalable. Plant cells have mechanisms for protein synthesis and posttranslational modification, including glycosylation, similar to those in animal cells. However, plants produce less complex and diverse Asn-attached glycans compared to animal cells and contain plant-specific residues. Nevertheless, plant-made antibodies (PMAbs) could be advantageous compared to those produced in animal cells due to the absence of a risk of contamination from nucleic acids or proteins of animal origin. In this review, the various platforms of PMAbs production are described, and the widely used transient expression system based on *Agrobacterium*-mediated delivery of genetic material into plant cells is discussed in detail. We examined the features of and approaches to humanizing the Asn-linked glycan of PMAbs, The prospects for PMAbs in the prevention and treatment of human infectious diseases have been illustrated by promising results with PMAbs against human immunodeficiency virus, rotavirus infection, human respiratory syncytial virus, rabies, anthrax and Ebola virus, The pre-clinical and clinical trials of PMAbs against different types of cancer, including lymphoma and breast cancer, are addressed. PMAb biosafety assessments in patients suggest that it has no side effects, although this does not completely remove concerns about the potential immunogenicity of some plant glycans in humans. Several PMAbs at various developmental stages have been proposed. Promise for the clinical use of PMAbs is aimed at the treatment of viral and bacterial infections as well as in anti-cancer treatment.

Increasing starch productivity of *Spirodela polyrhiza* by precisely control the spectral composition and nutrients status

Xu, YL; Tan, L; Guo, L; Yang, GL; Li, Q; Lai, F; He, KZ; Jin, YL; Du, AP; Fang, Y; Zhao, H (2019) INDUSTRIAL CROPS AND PRODUCTS 134: 284-291

Spectral composition and nutrients status are important environmental factors for biomass production. For realizing the economical and efficient production of duckweed [*Spirodela polyrhiza* (L.) Schleiden] turion, a promising starch-rich biofuel feedstock, impacts of tailored-lights combined with eutrophic/oligotrophic medium was investigated in this study. Results revealed that nutrients starvation was better for turion production and optimized spectral composition could significantly enhance it. Accompanied with simultaneously enhanced frond biomass and starch content, starch yield of 32.34 g/m^2 under fluorescent lamp was largely elevated to 51.71 g/m^2 by red/blue: 1/2. Especially, lights with higher ratio of red spectrum exerted strong inductive effect on turion formation under eutrophic condition. Considering the energy consumption, red/blue: 4/1 was the optimal option as it cut down energy cost by 12.56% and owned the highest production efficiency. Moreover, appropriate nutrients supplementation can further improve the starch productivity. Finally, an efficient and economical culture method for turion and starch production was established.

The influence of different plant hormones on biomass and starch accumulation of duckweed: A renewable feedstock for bioethanol production

Liu, Y; Chen, XY; Wang, XH; Fang, Y; Zhang, Y; Huang, MJ; Zhao, H (2019) RENEWABLE ENERGY 138: 659-665

Duckweed has been considered as a renewable feedstock for bioethanol production due to its fast generation of biomass and high starch accumulation. The use of plant hormones is a common and efficient method to manipulate plant growth and yield. However, the effects of different plant hormones on the biomass and starch accumulation of duckweed have not been systematically studied. Here, we screened five classes of plant hormones, including auxin, cytokinin, abscisic acid (ABA), gibberellins, and brassinosteroids. The results showed that the effect of 6-benzylaminopurine (6-BA, cytokinin) on promoting the biomass production of duckweed was much higher than other plant hormones. Compared with the control group, biomass yield at 1.0 x 10^{-3} mM 6-BA treated group increased by 37.41% in 7 days. Moreover, the results suggested that ABA can dramatically promote starch accumulation. The total starch that accumulated in the ABA treated samples was 3.3 times higher than that in the control samples. Taken together, these findings indicated that 6-BA and ABA were the most effective plant hormones in terms of enhancing biomass and starch accumulation. These results will provide valuable information for further studies of large-scale application of plant hormones in duckweed-to-bioethanol production in the future.

Salt Stress Induces Increase in Starch Accumulation in Duckweed (*Lemna aequinoctialis*, Lemnaceae): Biochemical and Physiological Aspects

de Morais, MB; Barbosa-Neto, AG; Willadino, L; Ulisses, C; Calsa, T (2019) JOURNAL OF PLANT GROWTH REGULATION 38: 683-700

In this study, antioxidant processes were searched for in macrophyte duckweed to investigate tolerance mechanisms in this species against oxidative damage caused by salinity stress. Biochemical and histological analyses were performed on four *Lemna aequinoctialis* clones grown in Schenk-Hildebrandt medium, 0.5xSH, supplemented with 1% sucrose liquid medium containing or not containing NaCl in different NaCl concentrations (0, 25 and 50 mM). For most clones, the salt stress effects caused growth inhibition and antioxidant responses at 50mM NaCl. Also, starch and reducing sugar accumulations were increased with salt, whereas the photosynthetic pigment content was reduced in clone *L. aequinoctialis* 5569. The plant growth inhibition reflects the oxidative stress shown by the significant increase in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content. In the *L. aequinoctialis* 5568 clone, with the highest MDA levels, no antioxidant enzymatic activity was observed. The *L. aequinoctialis* 5570 clone presented higher ascorbate peroxidase and catalase activities in parallel, indicating that the efficiency of the defence mechanism relies on synchrony between such enzyme activities toward successive elimination of reactive oxygen species and resulting in the assurance of some level of protection of the metabolism from oxidative damage. Considering the moderate salt stress (25mM), the maintenance of MDA content and small growth inhibition associated with the high starch production suggested the acclimation efficiency of *L. aequinoctialis* 5570 and 5567 clones, indicating that they may be suitable for cultivation under moderate saline conditions, serving as biofuel feedstock. In addition, this study demonstrates great intraspecific phenotypic plasticity of duckweed, *L. aequinoctialis*, from closely related clones.

A sustainable biorefinery approach for efficient conversion of aquatic weeds into bioethanol and biomethane

Kaur, M; Kumar, M; Singh, D; Sachdeva, S; Puri, SK (2019) ENERGY CONVERSION AND MANAGEMENT 187: 133-147

The study outlines an economical biorefinery concept to evaluate the potential of three aquatic weeds *Eichhornia crassipes*, *Lemna minor* and *Azolla microphylla* for co-production of ethanol and methane. Four different scenarios were investigated viz., scenario A-hydrothermal treatment followed by anaerobic digestion and ethanol fermentation respectively (HT -> AD -> EF), scenario B-thermochemical treatment followed by anaerobic digestion and ethanol fermentation respectively (TC -> AD -> EF), scenario C- hydrothermal treatment followed by ethanol fermentation and anaerobic digestion respectively (HT -> EF -> AD) and scenario D- thermochemical treatment followed by ethanol fermentation and anaerobic digestion respectively (TC -> EF -> AD). Sequential hydro thermal treatment and anaerobic digestion (HT -> AD) in scenario A enhanced the hemicellulose removal by 68.5-73.5% and simultaneously enriched the cellulose content by 41.2-54.5%. This contributed to highest ethanol yield (0.167-0.231 g/g biomass) in scenario A, which was found comparable to that obtained in scenario D, wherein harsh and expensive thermochemical pre-treatment was employed. Besides that, relatively higher methane yield of 209-257 dm^3/kg TOCR (Total organic carbon removal) obtained in scenario A as compared to other scenarios (67.5-238 dm^3/kg TOCR) improved the overall energy efficiency of the studied concept. The energetic assessment showed lowest total energy output (859.6-1322.7 kwh) in scenario B and C as compared to other scenarios (1041.8-1583.9 kwh), thereby confirming their incompetence in coupled bioenergy production. This study, therefore presents an economically and energetically sustainable approach for pre-treatment and bioenergy production that could help in overcoming the constraints hindering the commercialization of cellulosic ethanol. The study also opens up possibility for development of coupled aquatic weeds-based wastewater treatment and bioenergy production system for an efficient exploitation of the phytoremediation property of aquatic weeds.

Production of deuterated biomass by cultivation of *Lemna minor* (duckweed) in D_2O

Evans, BR; Foston, M; O'Neill, HM; Reeves, D; Rempe, C; McGrath, K; Ragauskas, AJ; Davison, BH (2019) PLANTA 249: 1465-1475

Common duckweed *Lemna minor* was cultivated in 50% D_2O to produce biomass with 50-60% deuterium incorporation containing cellulose with degree of polymerization close (85%) to that of H_2O-grown controls. The small aquatic plant duckweed, particularly the genus *Lemna*, widely used for toxicity testing, has been proposed as a potential source of biomass for conversion into biofuels as well as a platform for production of pharmaceuticals and specialty chemicals. Ability to produce deuterium-substituted duckweed can potentially extend the range of useful products as well as assist process improvement. Cultivation of these plants under deuterating conditions was previously been reported to require addition of kinetin to induce growth and was hampered by anomalies in cellular morphology and protein metabolism. Here, we report the production of biomass with 50-60% deuterium incorporation by long-term photoheterotrophic growth of common duckweed *Lemna minor* in 50% D_2O with 0.5% glucose. *L. minor* grown in 50% D_2O without addition of kinetin exhibited a lag phase twice that of H_2O-grown controls, before start of log phase growth at 40% of control rates. Compared to continuous white fluorescent light, growth rates increased fivefold for H_2O and

twofold for 50% D_2O when plants were illuminated at higher intensity with a metal halide lamp and a diurnal cycle of 12-h light/12-h dark. Deuterium incorporation was determined by a combination of H-1 and H-2 nuclear magnetic resonance (NMR) to be 40-60%. The cellulose from the deuterated plants had an average-number degree of polymerization (DPn) and polydispersity index (PDI) close to that of H_2O-grown controls, while Klason lignin content was reduced. The only major gross morphological change noted was root inhibition.

Duckweed as an Agricultural Amendment: Nitrogen Mineralization, Leaching, and Sorghum Uptake

Kreider, AN; Pulido, CRF; Bruns, MA; Brennan, RA (2019) JOURNAL OF ENVIRONMENTAL QUALITY 48: 469-475

Excessive N and P in surface waters can promote eutrophication (algae-dominated, low-O_2 waters), which decreases water quality and aquatic life. Duckweed (Lemnaceae), a floating aquatic plant, rapidly absorbs N and P from water and its composition shows strong potential as a soil amendment. Therefore, it may be used to transfer N and P from eutrophic water bodies to agricultural fields. In this work, dried duckweed was incorporated into agricultural soil in microcosm, column, and field tests to evaluate biological N cycling, nutrient retention, and crop yield compared with compost, diammonium phosphate (DAP), and an amendment-free control. In microcosm tests, 25 ± 13% of duckweed N was mineralized, providing on average less mineral N than DAP (107 ± 21%), but more than compost (11 ± 12%). In columns, duckweed treatments leached only 2% of the N added, significantly less than DAP, which leached 60% of its N. Compared with the control, DAP leached significantly more phosphate (78%), whereas duckweed and compost treatments leached less (56 and 27%, respectively). Crop yield, as well as runoff N and P, were measured in field tests growing forage sorghum [*Sorghum bicolor* (L.) Moench.]. Although less total N was applied to duckweed plots than to DAP plots (75 vs. 130 kg ha^{-1}), respectively), duckweed was found to retain 30% more total mineral N in a tilled agricultural field than DAP, while supporting a comparable yield. These tests indicate that duckweed may provide a sustainable source of N and P for agriculture.

Swelling behavior and satiating effect of the gel microparticles obtained from callus cultures pectins

Gunter, EA; Khramova, DS; Markov, PA; Popeyko, OV; Melekhin, AK; Beloserov, VS; Martinson, EA; Litvinets, SG; Popov, SV (2019) INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES 123: 300-307

Gel microparticles were prepared from pectins of campion (SVCgel) and duckweed (LMCgel) callus cultures, as well as from commercial apple pectin (APgel) by emulsion dehydration techniques with successive ionotropic gelation. The morphology and swelling behavior of the microparticles were determined after successive incubation in simulated gastric (SGF), intestinal (SIF), and colonic (SCF) fluids. Both SVCgel and LMCgel microparticles were found to swell in SGF and SIF gradually, and at oral administration decreased food intake by laboratory mice during the first 5 h of free-feeding. The SVCgel microparticles demonstrated the higher stability in SCF within 24 h than LMCgel ones. Only the SVCgel microparticles were shown to decrease food intake by 24% during the 21 h of free-feeding and decreased body weight of mice by 4% during 24 h after oral administration. The APgel microparticles lost their shape in SIF, then fully disintegrated after 0.5 h of incubation in SCF, and failed to affect food intake or mice body weight. The data obtained indicated that sustainability and

swelling of the gel microparticles from the SVC pectin in the colonic fluid may provide the stronger satiating effect compared to that of the LMCgel microparticles.

Ecology

Community composition and methane oxidation activity of methanotrophs associated with duckweeds in a fresh water lake

Iguchi, H; Umeda, R; Taga, H; Oyama, T; Yurimoto, H; Sakai, Y (2019) JOURNAL OF BIOSCIENCE AND BIOENGINEERING DOI:10.1016/j.jbiosc.2019.04.009

Methanotrophs are the only biological sink of the greenhouse gas methane. To understand the ecological features of methanotrophs in association with plants in the methane emitting environments, we investigated the community composition and methane oxidation of methanotrophs associated with duckweeds in a fresh water lake. Duckweeds collected from Lake Biwa, Japan over three summers showed methane consumption activity between 0.0067 and 0.89 μmol $h^{-1}g^{-1}$ (wet weight), with the highest values occurring from the end of July to August. The methanotrophic community on duckweeds consisted primarily of gamma-proteobacterial groups including the genera *Methylomonas* and *Methylocaldum*. Further analysis of co-cultures of a methanotroph isolate with sterilized duckweed revealed that the duckweed plant as well as the duckweed spent culture supernatant exerted an enhancing effect on methane oxidation. These results indicate that duckweeds not only provide a habitat for methanotrophs but also stimulate methanotrophic growth.

Negative frequency-dependent growth underlies the stable coexistence of two cosmopolitan aquatic plants

Armitage, DW; Jones, SE (2019) Ecology 100:e02657

Identifying and quantifying the mechanisms influencing species coexistence remains a major challenge for the study of community ecology. These mechanisms, which stem from species' differential responses to competition and their environments, promote coexistence if they give each species a growth advantage when rare. Yet despite the widespread assumption that co-occurring species stably coexist, there have been few empirical demonstrations in support of this claim. Likewise, coexistence is often assumed to result from interspecific differences in life-history traits, but the relative contributions of these trait differences to coexistence are rarely quantified, particularly across environmental gradients. Using two widely co-occurring and ecologically similar species of freshwater duckweed plants (*Spirodela polyrhiza* and *Lemna minor*), we tested hypotheses that interspecific differences in facultative dormancy behaviors, thermal reaction norms, and density-dependent growth promote coexistence between these species, and that their relative influences on coexistence change as average temperatures and fluctuations around them vary. In competition experiments, we found strong evidence for negative frequency-dependent growth across a range of both static and fluctuating temperatures, suggesting a critical role of fluctuation-independent stabilization in coexistence. This negative frequency dependence could be explained by our observation that for both species, intraspecific competition was over 1.5 times stronger than interspecific competition, granting each species a low-density growth advantage. Using an empirically parameterized competition model, we found that while coexistence was facilitated by

environmental fluctuations, fluctuation-independent stabilization via negative frequency dependence was crucial for coexistence. Conversely, the temporal storage effect, an important fluctuation-dependent mechanism, was relatively weak in comparison. Contrary to expectations, differences in the species' thermal reaction norms and dormancy behaviors did not significantly promote coexistence in fluctuating environments. Our results highlight how coexistence in two ubiquitous and ostensibly similar aquatic plants is not necessarily a product of their most obvious interspecific differences, and instead results from subtle niche differences causing negative frequency-dependent growth, which acts consistently on both species across environmental gradients.

The Fates of Nitrogen in an Experimental Wetland Food Web: a Stable Isotope Study

Hong, JM; Zhang, J; Ma, YT; Gu, BH; Lee, R (2019) WETLANDS 39: 303-310

Human activities have increased the availability of reactive nitrogen in many freshwater ecosystems, leading to negative impacts on the health and biodiversity of lakes, rivers and wetlands. Yet, understanding the pathways of nutrient cycling in wetlands is limited. We conducted a nitrogen stable isotope (N^{15}) tracer study to assess nitrogenous nutrient transfers within the biota community in a small experimental wetland between August 13, 2016, and September 12, 2016. Results showed rapid use of nitrogen by various aquatic plants and subsequent transfer to the consumer community. N^{15} enrichments were found in all plant samples from the first collection event. The fastest nutrient uptake was found in the simplest form of plants (*Periphyton* and *Lemna minor*), followed by submerged macrophytes (*Ceratophyllum demersum* and *Utricularia vulgaris*). Emergent plants displayed the lowest N^{15} enrichment, likely due to their inability to directly assimilate nutrients from the water column and their initial large biomass. N^{15} enrichments in consumers were also pronounced, indicating that the nitrogen added to the wetland surface water was quickly transferred to various trophic levels. Primary consumers were more strongly labelled than secondary consumers; water column feeders were typically more enriched in N^{15} than benthic consumers. This experiment revealed different uses of nitrogen among the plant and consumer communities, and the results may provide useful information on community secession and ecosystem restoration under anthropogenic influence.

Ecology of *Stratiotes aloides* L. (Hydrocharitaceae) in Eurasia

Efremov, AN; Sviridenko, BF; Toma, C; Mesterhazy, A; Murashko, YA (2019) FLORA 253: 116-126

Stratiotes aloides (Hydrocharitaceae) is a member of hydromacrophyte communities, which are widespread in the waterbodies of Eurasia. In the course of our study, which covered both fieldwork across a large part of the species range as analysis of published sources, we determined that *S. aloides* is a Pliocene relic showing a boreal-sublatitudinal sub-Eurasian range with a small North American enclave. Its populations are generally quite stable across much of their current range. However, in Western, Central and southern Europe and in the South of European Russia, both local population sizes and area of occupancy of *S. aloides* populations decline significantly, mainly due to habitat degradation. Regeneration via seed is of particular importance to the plant in extreme conditions, and also for maintaining or increasing the genetic diversity of its populations. Seeds are usually more easily dispersed, across a wider area, than vegetative propagules produced by the plant. Yet, in some populations sexual reproduction is hindered by spatial isolation of the plants of different sexual types and prevalence of gynomonoecious populations. On the other hand, vegetative (clonal) propagation of *S. aloides* can result in locally-high biological productivity, and extensive

occupation of water area by the plant at individual sites. Populations of *S. aloides* have been found to co-occur with at least 134 other macrophyte species variously present. Highly constant species (present in more than 50% of communities containing *S. aloides*) include *Ceratophyllum demersum*, *Hydrocharis* morsus-ranae, *Lemna trisulca*, *Potamogeton perfoliatus, Spirodela polyrhiza*, and *Utricularia vulgaris*. This species prefers mesotrophic, beta-mesosaprobic freshwaters, and detrital, muddy-clayey bottom soils.

Influence of physicochemical water quality on aquatic macrophyte diversity in seasonal wetlands

Rameshkumar, S; Radhakrishnan, K; Aanand, S; Rajaram, R (2019) APPLIED WATER SCIENCE 9: UNSP 12

The present study aims to assess the physicochemical parameters and distribution of aquatic macrophytes of seasonal wetlands flowing into the coast of Palk Bay, southeast coast of India. We tested the hypothesis whether there is any statistically significant difference in physicochemical parameters and macrophyte communities among study location. Water quality parameters such as temperature, pH, salinity, TDS, DO, turbidity and electrical conductivity, and the aquatic macrophyte diversity were estimated in all three stations. One-way analysis of variance (ANOVA) and Pearson correlation were employed to assess the relationship between water quality parameters, and the water quality index (WQI) was computed to assess the status of water conditions. ANOVA revealed that there is no statistically significant difference ($p>0.05$) in water quality parameters among the three stations. The lowest f value 0.180 was recorded for pH and highest of 2.478 for TDS. A total of 7 submerged macrophytes, namely *Ceratophyllum demersum* L., *Egeria densa* Planch., *Lemna minor* L., *Marsilea quadrifolia* L., *Sagittaria guayanensis* and *Isoetes riparia*; 6 rooted floating weeds, namely *Potamogeton nodosus* Poir., *Nymphaea odorata* Aiton., *Nelumbo nucifera* Gaertn., *Myriophyllum spicatum* L. and *Hydrilla verticillata*; 1 floating, namely *Eichhornia crassipes* Kunth; and 1 rooted macrophyte, namely *Najas minor*, were recorded in Tharavai wetland. A deplorable water quality condition was found in the selected study area, which was evidenced from WQI (>76%). Of this, submerged aquatic vegetation is used as the water quality key indicator, and it exists where there is a better water quality condition. The EC, TDS and turbidity negatively influenced the aquatic macrophytes. Therefore, there is a need for some adaptation measure to maintain the water quality for more extended period for domestic use.

Factors Influencing Macrophyte Species Richness in Unmodified and Altered Watercourses

Hachol, J; Bondar-Nowakowska, E; Nowakowska, E (2019) POLISH JOURNAL OF ENVIRONMENTAL STUDIES 28: 609-622

The aim of this study was to determine the link between the elements of a riverbed system (river length, longitudinal profile, bottom width, river bed depth, bank slope, bank protection, bottom substrate, and level of silt build-up), modified by regulatory and maintenance work, the number of aquatic vascular plant species, and their evenness present in both small and medium lowland streams in Poland. 100 study sections were analysed in 29 watercourses. Due to their geological, hydromorphological, and climate settings, the examined watercourses are representative of the central European plains, of which 65 sections are located in regulated and maintained watercourses and 35 are in unmodified streams. The Shapley value regression method was used to establish the

influence of the stream features on aquatic plants. Results identified 27 different macrophyte taxa, where the most frequently occurring were *Sparganium emersum* Rehmann, *Phalaris arundinacea* L., and *Lemna minor* L. The results found that aquatic plant communities were influenced by the analysed factors, regardless of whether a watercourse was shaped by technical means or not. The most influential parameters were the level of silt build-up and bottom width. Furthermore, results brought to evidence that watercourses are complex systems where elements are linked by a series of relationships and that single correlations among environmental elements, anthropogenic interactions, and aquatic plants are very rare.

Feed & Food

Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture – A review

Sonta, M; Rekiel, A; Batorska, M (2019) ANNALS OF ANIMAL SCIENCE 19: 257-271

The aim of this study was to determine the possibility of using duckweed in sustainable livestock production and aquaculture. Duckweed is a small plant which grows in water and is exploited in biotechnology, dietetics, phytotherapy, and ecotoxicology. It is also used for biological wastewater treatment, and for biogas and ethanol production. This study provides the characteristics of duckweed and presents results indicating its applicability in livestock feeding. Duckweed is a rich source of proteins and amino acids, and contains many macro- and micronutrients as well as vitamins and carotenoids. Unfortunately, it accumulates considerable amounts of toxic metals and compounds from the aquatic environment, which may limit its use as a feed ingredient. Fresh or dried duckweed is willingly consumed by animals (poultry - laying hens, broiler chickens, ducks; cows, sheep, goats, swine, fish) and is a valuable protein source to them. It has been scientifically demonstrated that its use in moderate amounts or as a partial replacement of other protein feed materials, including soybean meal, has a beneficial effect on the productivity, fattening, and slaughter performance of livestock and poultry as well as on the quality of their meat and eggs. Research addressing duckweed use as a feed ingredient should focus on developing various growth media technologies, including the use of slurry digestate, to obtain high biomass yields. Another research direction should be to determine risks in the production chain (collection, processing), which limit its use in monogastric and ruminant diets.

Slurry-grown duckweed (*Spirodela polyrhiza*) as a means to recycle nitrogen into feed for rainbow trout fry

Stadtlander, T; Forster, S; Rosskothen, D; Leiber, F (2019) JOURNAL OF CLEANER PRODUCTION 228: 86-93

Liquid manure from livestock production systems is a major source of nitrogen and phosphorus release from nutrient cycles and a cause of ecosystem eutrophication. Duckweeds, small aquatic plants, may be used to recover N and P from livestock slurry while producing high-quality protein feed. In order to assess N and P uptake efficiency and utility for fish feed, two duckweed species, *Landoltia punctata* and *Spirodela polyrhiza*, were grown in controlled climate chambers on two nutrient-rich media: diluted (1:10) cattle slurry and mechanically filtered household sewage. Treatments were in triplicate, each running in four cycles with fresh substrate (one week each).

Spirodela polyrhiza exhibited the strongest growth (96 g fresh matter m^{-2} day^{-1}) and highest protein content (306 g per kg dry matter) on diluted slurry. The weakest growth was found for *L. punctata* on treated sewage (52 g fresh matter m^{-2} d^{-1}). Average removal of total provided and utilizable inorganic N from the media was 73.2% and 83.9% for sewage and diluted slurry, respectively. *Spirodela polyrhiza* grown on diluted slurry was subsequently tested as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fry. Two different ingredient levels of *S. polyrhiza* meal (6.25% and 12.5% of feed) were fed to rainbow trout fry for 4 weeks, during which fish growth, feed and nutrient utilization and gut health were assessed. Feed was accepted, but both duckweed meal treatments resulted in 5-10% poorer growth traits and feed efficiency compared to control. The intestine somatic index was not affected. This is the first time the potential of duckweed as feed for rainbow trout fry has been demonstrated. Furthermore, our experiments found considerable N and P uptake from diluted slurry by *S. polyrhiza*, which produced protein at a high rate per unit time and area.

A Green-Mediterranean Diet, Supplemented with Mankai Duckweed, Preserves Iron-Homeostasis in Humans and Is Efficient in Reversal of Anemia in Rats

Meir, AY; Tsaban, G; Zelicha, H; Rinott, E; Kaplan, A; Youngster, I; Rudich, A; Shelef, I; Tirosh, A; Brikner, D; Pupkin, E; Sarusi, B; Bluher, M; Stumvoll, M; Thiery, J; Ceglarek, U; Stampfer, MJ; Shai, I (2019) JOURNAL OF NUTRITION 149: 1004-1011

Decreased dietary meat may deplete iron stores, as plant-derived iron bioavailability is typically limited. We explored the effect of a low-meat Mediterranean (green-MED) diet, supplemented with *Wolffia globosa* duckweed (Mankai: rich in protein and iron) as a food source for humans, on iron status. We further examined the iron bioavailability of Mankai in rats. Two hundred and ninety-four abdominally obese/dyslipidemic [mean age = 51.1 y; body mass index (kg/m2) = 31.3; 88% men] nonanemic participants were randomly assigned to physical activity (PA), PA + MED diet, or PA + green-MED diet. Both isocaloric MED groups consumed 28 g walnuts/d and the low-meat green-MED group further consumed green tea (800 mL/d) and Mankai (100 g green shake/d). In a complementary animal experiment, after 44 d of an iron deficiency anemia-inducing diet, 50 female rats (age = 3 wk; Sprague Dawley strain) were randomly assigned into: iron-deficient diet (vehicle), or vehicle + iso-iron: ferrous gluconate (FG) 14, Mankai 50, and Mankai 80 versions (1.7 $mg.kg^{-1}d^{-1}$ elemental iron), or FG9.5 and Mankai 50-C version (1.15 $mg.kg^{-1}d^{-1}$ elemental iron). The specific primary aim for both studies was changes in iron homeostasis parameters. After 6 months of intervention, iron status trajectory did not differ between the PA and PA + MED groups. Hemoglobin modestly increased in the PA + green-MED group (0.23 g/dL) compared with PA (-0.1 g/dL; $P < 0.001$) and PA + MED (-0.1 g/dL; $P < 0.001$). Serum iron and serum transferrin saturation increased in the PA + green-MED group compared with the PA group (8.21 μg/dL compared with -5.23 μg/dL and 2.39% compared with -1.15%, respectively; $P < 0.05$ for both comparisons), as did folic acid ($P = 0.011$). In rats, hemoglobin decreased from 15.7 to 9.4 mg/dL after 44 d of diet-induced anemia. After depletion treatment, the vehicle-treated group had a further decrease of 1.3 mg/dL, whereas hemoglobin concentrations in both FG and Mankai iso-iron treatments similarly rebounded (FG14: + 10.8 mg/dL, Mankai 50: + 6.4 mg/dL, Mankai 80: + 7.3 mg/dL; FG9.5: + 5.1 mg/dL, Mankai 50-C: + 7.1 mg/dL; $P < 0.05$ for all vs. the vehicle group). In humans, a green-MED low-meat diet does not impair iron homeostasis. In rats, iron derived from Mankai (a green-plant protein source) is bioavailable and efficient in reversal of anemia. This trial was registered at clinicaltrials.gov as NCT03020186.

Duckweed for Human Nutrition: No Cytotoxic and No Anti-Proliferative Effects on Human Cell Lines

Sree, KS; Dahse, HM; Chandran, JN; Schneider, B; Jahreis, G; Appenroth, KJ (2019) PLANT FOODS FOR HUMAN NUTRITION 74: 223-224

Duckweeds (Lemnaceae) possess good qualitative and quantitative profiles of nutritional components for its use as human food. However, no studies have been conducted on the probable presence or absence of any adverse effects. The extracts from seven duckweed species (*Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Lemna minor, Wolffiella hyalina, Wolffia globosa*, and *Wolffia microscopica*) covering all five genera of the plant family were herewith tested for cytotoxic effects on the human cell lines HUVEC, K-562, and HeLa and for anti-proliferative activity on HUVEC and K-562 cell lines. From these assays, it is evident that duckweeds do not possess any detectable anti-proliferative or cytotoxic effects, thus, the high nutritional value is not diminished by such detrimental factors. The present result is a first step to exclude any harmful effects of highly nutritious duckweed for human.

Evaluation of live duckweed, *Wolffia globosa* as an allochthonous feed for *Labeo rohita* fry during nursery rearing

Pradhan, A; Patel, AB; Singh, SK (2019) AQUACULTURE RESEARCH 50: 1557-1563

A 40 days feeding trial with *Labeo rohita* (rohu) fry was conducted in six outdoor cemented tanks (5m x 4m x 1 m; 6-8 cm soil base) to establish the relative impact of on-farm grown live *Wolffia globosa* (a duckweed with crude protein of 28.57%) and a formulated iso-nitrogenous feed (crude protein 28.37%) on growth performance, survival, health and overall quality of fingerlings. The experimental tanks were dried, limed at 250 kg/ha, filled with ground water and subsequently applied with cow dung (soaked overnight) and mustard oil cake at 1,000 kg/ha and 50 kg/ha respectively. The fry were stocked at 0.3 millions/ha in six prepared tanks, and fed live *Wolffia* (T1) and formulated feed (T2). Results of the study indicate better performance ($p < 0.05$) of fry in terms of final mean weight (7.83 g), length (8.82 cm), daily growth index (6.51 g %/day) and survival (89.5%) than those fed on formulated feed. Furthermore, significantly improved ($p < 0.05$) feed utilization parameters viz. apparent feed conversion ratio (AFCR), apparent protein conversion efficiency (APCE), apparent protein efficiency ratio (APER); haematological parameters viz. total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), plasma glucose and protein of fry were also observed in T1 (live Wolffia). Overall findings from this study hints towards the possible utilization of on-farm grown live *Wolffia* as a suitable nutrient source for fry rearing of *L. rohita*.

Interaction with other organisms

First Nearctic record of *Hydrilla albilaris* (Meingen) (Diptera: Ephydridae), a leaf miner of duckweed (Araceae: *Lemnoideae*), with comments on related species

Eiseman, CS; Zatwarnicki, T (2019) PROCEEDINGS OF THE ENTOMOLOGICAL SOCIETY OF WASHINGTON 121: 160-167

Hydrellia albilabris (Meigen) (Diptera: Ephydridae) is recorded in the Nearctic Region for the first time. Its biology and immature stages are documented, and other miners in duckweeds (Araceae: *Lemnoideae*) are discussed. One new combination is proposed: *Hydrellia jinpingensis* (Zhang, Yang, and Hayashi) is transferred from *Cavatorella*.

How does *Stratiotes abides* L. affect the growth and turion formation of *Spirodela polyrhiza* (L.) Schleiden?

Strzalek, M; Kufel, L; Wysokinska, U (2019) AQUATIC BOTANY 154: 45-52

Stratiotes abides L. (water soldier) is a large representative of Hydrocharitaceae family, which in summer produces a large biomass floating on the water and is known for high demands for space or nutrients and for its allelopathic activity. We examined the effects of this macrophyte on the water in its stands, specifically N and P concentrations, and on resulting effects on the internal N and P concentrations, the relative growth rates (RGR), colony disintegration and turion formation of the free-floating *Spirodela polyrhiza* in outdoor ex-situ experiments. Natural populations of duckweeds accompanying dense, extensive Stratiotes stands or growing in water bodies free of *Stratiotes* were cultivated for short periods in original water or in this water enriched with ammonium and phosphates. Waters from Stratiotes stands were poorer in dissolved inorganic nitrogen concentrations but did not decrease tissue N concentrations, RGR or colony sizes of *Spirodela*. Nutrient enrichment and *Spirodela* origin neither affected RGR nor colony disintegration of *Spirodela*. In a long-term experiment, *Stratiotes* stand water caused a shift in duckweed developmental processes from vegetative frond production to turion formation, manifested mainly by high total number of turions and high number of turions per frond at the end of the long-term experiment and by the fast rate of turion formation. Further, it seems that this water exerted a long-lasting effect on neighbouring populations of *Spirodela* and shortened their lifespan.

Combination of heterotrophic nitrifying bacterium and duckweed (*Lemna gibba* L.) enhances ammonium nitrogen removal efficiency in aquaculture water via mutual growth promotion

Shen, M; Yin, Z; Xia, D; Zhao, Q; Kang, Y (2019) THE JOURNAL OF GENERAL AND APPLIED MICROBIOLOG DOI:10.2323/jgam.2018.08.002

We created a combined system using duckweed and bacteria to enhance the efficiency of ammonium nitrogen (NH_4^+-N) and total nitrogen (TN) removal from aquaculture wastewater. Heterotrophic nitrifying bacterium was isolated from a sediment sample at an intensive land-based aquaculture farm. It was identified as *Acinetobacter* sp. strain A6 based on 16S rRNA gene sequence (accession number MF767879). The NH_4^+-N removal efficiency of the strain and duckweed in culture media and sampled aquaculture wastewater at 15°C was over 99% without any accumulation of nitrite or nitrate. This was significantly higher than strain A6 or duckweed alone. Interestingly, the presence of NO_3^- increased NH_4^+-N removal rate by 35.17%. Strain A6 and duckweed had mutual growth promoting-effects despite the presence of heavy metals and antibiotics stresses. In addition, strain A6 colonized abundantly and possibly formed biofilms in the inner leaves of duckweed, and possessed indoleacetic acid (IAA)- and siderophore-producing characteristics. The mutual growth promotion between strain A6 and duckweed may be the reason for their synergistic action of N removal.

"Duckweed-Microbe Co-Cultivation Method" for Isolating a Wide Variety of Microbes Including Taxonomically Novel Microbes

Tanaka, Y; Tamaki, H; Tanaka, K; Tozawa, E; Matsuzawa, H; Toyama, T; Kamagata, Y; Mori, K (2019) MICROBES AND ENVIRONMENTS 33: 402-406

We herein described a new microbial isolation method using the interaction between the floating aquatic plant, duckweed, and microbes. We harvested microbial cells from Japanese loosestrife roots and co-cultivated these cells with aseptic duckweed using artificial inorganic medium for the plant for four weeks. During the co-cultivation, some duckweeds were collected every week, and the roots were used for microbial isolation using a low-nutrient plate medium. As a result, diverse microbial isolates, the compositions of which differed from those of the original source (Japanese loosestrife root), were obtained when the roots of duckweed were collected after 2 weeks of cultivation. We also successfully isolated a wide variety of novel microbes, including two strains within the rarely cultivated phylum, Armatimonadetes. The present study shows that a duckweed-microbe co-cultivation approach together with a conventional technique (direct isolation from a microbial source) effectively obtains more diverse microbes from a sole environmental sample.

Molecular Biology

Analysis of codon usage in the mitochondrion genome of *Spirodela polyrhiza*

Huang, MX; Zhong, YS; Ma, XY; Hu, QX; Fu, MH; Han, YL (2019) AQUATIC BOTANY 156: 65-72

In this study, investigation into the characteristics of codon bias and factors in shaping the codon usage pattern of the mitochondrial genome of *Spirodela polyrhiza* L. Schleid (strain 7498) was performed. *S. polyrhiza* had a very high GC content of 45.7% in the mitochondrial genome. Compared with other monocots whose mitochondrial genomes have been sequenced, the GC contents of mitochondrial genome of monocots seem to have a decreasing trend as the molecular evolution, and then finally trend to stable at about 44%. The mitochondrion of *S. polyrhiza* had extremely narrow GC(3s) distributions from 0.212 to 0.433, if taking no account of SpMatR (0.559) which was the only protein-coding genes with G/C-biased codon usages. The neutrality plot (GC_{12} vs. GC_{3s}) indicated that there was no correlation between GC_{12} and GC_{3s}, suggesting there was little directional mutation pressure on the mitochondrial genome of *S. polyrhiza*. The ENC-plot (ENC vs. GC_{3s}) showed that there were only a small number of genes lying on the expected curve, but a majority of the points with low ENC values lied well below the expected curve. According to the frequency distributions of the ENC ratio, the dataset displayed a single peak, and the most of genes located into a narrow region of ENC ratios between 0-0.1. Context-dependent mutation was analyzed by Chi square test for contingency tables at the four fold degenerate (FFD) sites, suggesting that the frequencies of N_3-position bases were definitely not independent of the N_2-position bases. Correspondence analysis (COA) of relative synonymous codon usage (RSCU) yielded a first axis which accounted for only13.23% of the total variation. These results suggested that although other factors might play a role in the mitochondrion evolution of *S. polyrhiza*, mutation press still was the major factor in shaping the codon usage. Meantime, six codons including UUA(Leu), CAU(His), CGU(Arg), AUU(Ile), GCU(Ala) and GAA(Glu) were determined as the "optimal codons" (p <

0.01). Among them, the codon UUA(Leu), its usage was significantly more often in highly biased genes than in lowly.

Variation in genome size, cell and nucleus volume, chromosome number and rDNA loci among duckweeds

Hoang, PTN; Schubert, V; Meister, A; Fuchs, J; Schubert, I (2019) SCIENTIFIC REPORTS 9: 3234

Duckweeds are small, free-floating, largely asexual and highly neotenous organisms. They display the most rapid growth among flowering plants and are of growing interest in aquaculture and genome biology. Genomic and chromosomal data are still rare. Applying flow-cytometric genome size measurement, microscopic determination of frond, cell and nucleus morphology, as well as fluorescence in situ hybridization (FISH) for localization of ribosomal DNA (rDNA), we compared eleven species, representative for the five duckweed genera to search for potential correlations between genome size, cell and nuclei volume, simplified body architecture (neoteny), chromosome numbers and rDNA loci. We found a similar to 14-fold genome size variation (from 160 to 2203 Mbp), considerable differences in frond size and shape, highly variable guard cell and nucleus size, chromosome number (from 2n = 36 to 82) and number of 5S and 45S rDNA loci. In general, genome size is positively correlated with guard cell and nucleus volume ($p < 0.001$) and with the neoteny level and inversely with the frond size. In individual cases these correlations could be blurred for instance by particular body and cell structures which seem to be linked to specific floating styles. Chromosome number and rDNA loci variation between the tested species was independent of the genome size. We could not confirm previously reported intraspecific variation of chromosome numbers between individual clones of the genera *Spirodela* and *Landoltia*.

Gene coexpression analysis reveals dose-dependent and type-specific networks responding to ionizing radiation in the aquatic model plant *Lemna minor* using public data

Fu, LL; Ding, ZH; Kumpeangkeaw, A; Sun, XP; Zhang, JM (2019) JOURNAL OF GENETICS 98: 9

Ionizing radiations (IRs) are widespread damaging stresses to plant growth and development. However, the regulatory networks underlying the mechanisms of responses to IRs remains poorly understood. Here, a set of publicly available transcriptomic data (conducted by Van Hoeck et al. 2015a), in which *Lemna minor* plants were exposed to a series of doses of gamma, beta and uranium treatments was used to perform gene coexpression network analysis. Overall, the genes involved in DNA synthesis and chromatin structure, light signalling, photosynthesis, and carbohydrate metabolism were commonly responsive to gamma, beta and uranium treatments. Genes related to anthocyanin accumulation and trichome differentiation were specifically downregulated, and genes related to nitrogen and phosphate nutrition, cell vesicle transport, mitochondrial electron transport and ATP synthesis were specifically upregulated in response to uranium treatment. While genes involved in DNA damage and repair, RNA processing and RNA binding were specifically downregulated and genes involved in calcium signalling, redox and degradation of carbohydrate metabolism were specifically upregulated responding to gamma radiation. These findings revealed both dose-dependent and type-specific networks responding to different IRs in *L. minor*, and can be served as a useful resource to better understand the mechanisms of responses to different IRs in other plants.

Physiology

In vitro proliferation of Lebanese *Lemna minor* and *Lemna gibba* on different nutrient media

Ghanem, H; Haddad, A; Baydoun, S; Abou Hamdan, H; Korfali, S; Chalak, L (2019) JOURNAL OF TAIBAH UNIVERSITY FOR SCIENCE 13: 497-503

This study aimed to assess the ability of two Lebanese duckweed species, (*Lemna minor* and *L. gibba*), to grow under in vitro conditions on three nutritive solutions, Murashige Skoog (MS), Schenk-Hildebrand (SH) and Algal Assay Procedure (AAP). Plant growth of both species, expressed as doubling time, differed significantly ($p<.05$) between the tested media with best results obtained on SH for *L. minor* after 7 days of culture and on AAP for *L. gibba* after 2.7 days for both species. Growth index was significantly higher ($P<.05$) for *L. minor* on SH reaching 41.6 after 21 days of culture whereas *L. gibba* exhibited its highest growth index of 15.51 on AAP. These results indicate the efficiency of SH and AAP media in promoting the vegetative proliferation of *L. minor* and *L. gibba* to further provide a good source of duckweed material for phytoremediation applications.

Phytomedicine

The effect of *Wolffia globosa* Mankai, a green aquatic plant, on postprandial glycemic response: A randomized crossover controlled trial

Zelicha, H; Kaplan, A; Meir, AY; Tsaban, G; Rinott, E; Shelef, I; Tirosh, A; Brikner, D; Pupkin, E; Qi, L; Thiery, J; Stumvoll, M; Kloting, N; von Bergen, M; Ceglarek, U; Bluher, M; Stampfer, MJ; Shai, I (2019) DIABETES CARE 42: 1162-1169

To compare the postprandial and overnight glycemic response using a novel green aquatic plant thought to provide a dietary source for high-quality protein, with an iso-carbohydrate/protein/caloric dairy shake. This is a randomized controlled crossover trial among 20 abdominally obese participants (age 51.4 years; fasting plasma glucose 110.9 mg/dL), who were allocated to replace dinner with either, first, a green shake containing *Wolffia globosa* duckweed (Mankai: specific-strain) or an iso-carbohydrate/protein/calorie yogurt shake. A 2-week flash glucose-monitoring system was used to assess postmeal glucose dynamics (6 net administration days; 97 observation days in total). We further obtained from each participant dietary/daily activity/satiety scale/sleep logs. Participants were recruited from the green-Mediterranean diet arm of the 18-month Dietary Intervention Randomized Controlled Trial-Polyphenols Unprocessed (DIRECT-PLUS) study. *Wolffia globosa* Mankai elicited a lower postprandial glucose peak compared with yogurt (increment peak = 13.4 ± 9.2 vs. 19.3 ± 15.1 mg/dL; P = 0.044), which occurred later (77.5 ± 29.2 vs. 59.2 ± 28.4 min; P = 0.037) and returned faster to baseline glucose levels (135.8 ± 53.1 vs. 197.5 ± 70.2 min; P = 0.012). The mean post-net incremental area under the curve (netAUC) was lower with *Wolffia globosa* up to 60 and 180 min (netAUC 60 min: 185.1 ± 340.1 vs. 441.4 ± 336.5 mg/dL/min, P = 0.005; netAUC 180 min: 707.9 ± 1,428.5 vs. 1,576.6 ± 1,810.1 mg/dL/min, P = 0.037). A *Wolffia globosa*-based shake replacing dinner resulted in lower next-morning fasting glucose levels (83.2 +/- 0.8 vs. 86.6 +/- 13 mg/dL; P = 0.041). Overall, postprandial glucose levels from the shake administration until the next morning were lower in the *Wolffia globosa* Mankai green shake compared with the yogurt shake (P < 0.001). Overnight sleep duration was similar (378.2 ± 22.4 vs. 375.9 ±28.4 min; P = 0.72), and satiety

rank was slightly higher for the *Wolffia globosa* shake compared with the yogurt shake (7.5 vs. 6.5; P = 0.035). *Wolffia globosa* Mankai duckweed may serve as an emerging alternative plant protein source with potential beneficial postprandial glycemic effects.

Pharmacotherapeutic action analysis of mineral substances of medical plants, which are used in thyroid gland disease

Vladymyrova, I; Georgiyants, V; Savelieva, E (2019) BULLETIN OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN 1: 6-13

Diseases of thyroid gland refer to the most widely spread ones, and are accompanied by the change of hormonal background of the human organism in the whole and lead to disorders of vitally important processes in other organs, triggering appearance of concomitant diseases. Among major causes of thyroid gland diseases are psychological and emotional overloads; malnutrition and unbalanced nutrition that results in deficiency of mineral substances and vitamins in the organism; radioactive conditions and unfavorable ecological situations; chronic diseases. Taking into account such a great role of macro- and microelements in the normal work of the thyroid gland, it is necessary to provide their physiologically necessary content in the organism. The sources of mineral substances can be medicinal plants and remedies made of them. Therefore, the aim of the work was to determine content of macro- and microelement composition of medicinal plants - *Lycopus* herb, *Feijoa* leaves, *Cetraria islandica* thalli, Bugleweed European herb, *Genista* herb, leaves of *Lemna minor*, thalli of *Fucus vesiculoses* and thalli of *Laminaria*, which are used in treatment of thyroid gland diseases and their water extracts and 10 %, 30 % and 50 % alcohol tinctures. Quantitative content determination of macro- and microelements has been carried out by the atomic emission spectrography method. Quantitative determination of general iodine content has been performed by the iodometry method (titrant - 0.01 M sodium thiosulphate solution) after preliminary burning of the raw material in the alkaline solution by the methodology of State Pharmacopoeia of Ukraine 2.0 volume 3, monograph "Buri vodorosli". The presence of 15 macro and microelements has been determined in the result of the performed spectral analysis of the studied types of medicinal plant raw materials and their extracts. Silicium, mangan, magnesium, calcium, sodium and potassium are contained in comparatively big amounts. When determining qualitative and quantitative content of mineral substances our attention was focused on the elements which had great significance at diseases of thyroid gland, the deficiency or misbalance, which can damage the thyroid gland or other organs functioning, the use of which can eliminate negative symptomatic manifestations in diseases of thyroid gland (Fe, Mn, Mg, Ca, Cu, Zn, Se, I). The representatives of brown algae - *Laminaria* (0,11 %) and *Fucus* (0,05 %) and also the representative of fresh water reservoirs - duckweed (0,028 %) are characterized by the highest iodine content. The samples of *Laminaria, Fucus, Feijoa* and *Lemna* (duckweed) were characterized by the highest iodine content. The most maximum iodine content had water extracts, when ethyl alcohol concentration was increased, the iodine content didn't change significantly. High selenium content has been determined in the thalli of *Laminaria* (0,81 mg/kg), *Lemna* (duckweed) (0,72 mg/kg) and fresh *Feijoa* fruit (0,31 mg/kg). The highest selenium content (mkg/l) had water *Fucus* extract (40) and 50 % *Lemna* (duckweed) tincture (14). There is an interesting fact, that iodin: selenium ratio in these samples was 1:4(5), that can stipulate similar mechanisms of the effect for thyroid gland. Among the tested substances high selenium and iodine content had simultaneously *Laminaria* samples - 5 % tincture (3,8) and 10 % tincture (6,6). Though the iodine: selenium ratio had another character and made 18(19):1.

Phytoremediation

Phytoremediation of arsenic-contaminated water by *Lemna valdiviana*: An optimization study.

Souza, TD de; Borges, AC; Braga, AF; Veloso, RW; Teixeira de Matos, A)2019) CHEMOSPHERE 234: 402-408

Phytoremediation is a technique in which plants are used to treat contaminated media. The objective of this study was to monitor the influence of the parameters pH, phosphate concentration, and nitrate concentration in the process of arsenic absorption by *Lemna valdiviana* Phil. The response surface methodology was used to analyze the data to subsidize actions that maximize the phytoremediation process. A central composite rotational design (CCRD) was used with 3 variables including 6 axial points and 6 repetitions at the central point, totalling 20 trials. The plants were exposed to a constant concentration of arsenic in the optimization test of 0.5 mg L^{-1} ($NaAsO_2$) and varied levels of pH, P-PO_4, and N-NO_3 in a period of 7 d. At the end of the experiment, the mass of arsenic removed from water and arsenic accumulated in the plants, the arsenic species present, the relative growth rate of plants (RGR), the tolerance index (TI), and the bioaccumulation factor (BAF) were calculated. *Lemna valdiviana* absorbed a greater amount of As when cultivated under pH conditions between 6.3 and 7.0, readily available phosphorus (P-PO_4) concentration of 0.0488 mM, and nitrogen in the form of 7.9 mM nitrate. Under these conditions, the plants were able to accumulate 1190 mg kg^{-1} As (in dry weight) from the aqueous media and reduce 82% of its initial concentration. Therefore, *Lemna valdiviana* has been shown to be an arsenic bioaccumulating macrophyte with high phytoremediation potential for media contaminated with the metalloid.

Phytoremediation performance of *Lemna* communities in a constructed wetland system for wastewater treatment

Ceschin, S; Sgambato, V; Ellwood, NTW; Zuccarello, V (2019) ENVIRONMENTAL AND EXPERIMENTAL BOTANY 162: 67-71

Duckweeds are considered suitable plants for bioremediation of wastewaters because they can tolerate and take up diverse pollutants and large quantities of nutrients. They have year-round vegetative growth that can reach very high rates using wastewater nutrients that can accumulate intracellularly or be utilized to produce new biomass. The constructed wetland that services the town of Forano (Central Italy) is composed of three interconnected, but distinct, treatment pools (P1, P2, P3). In this study the phytoremediation performance of the whole treatment system was assessed taking into consideration pH, temperature, oxygen, nitrates, phosphates, sulphates, and pathogenic bacteria (*Escherichia coli*) at the inlet and outlet of each pool once per season over a year. The remediation efficiency of P2 was lower than in P1 and P3, and this was shown to be due to an extended, dense free-floating *Lemna* mat. The mat was thick enough (18-30 mm) to block light penetration, and heat and gas exchange to the underlying water resulting in dark, cool and near-anaerobic conditions. These conditions limited aerobic biodecomposition processes and natural control of the *E. coli* population. The underlying fronds of the *Lemna* mat in P2 were highly necrotic and became a source of nutrients, shown by an increase in nitrates and phosphates from the inlet to the outlet. *Lemna* use in phytoremediation is often considered highly important, but it is shown here

that active management interventions are required and should involve periodic harvesting of *Lemna* fronds to avoid mat over-development and formation of necromass.

Cost-effectiveness of boron (B) removal from irrigation water: an economic water treatment model (EWTM) for farmers to prevent boron toxicity

Turker, OC; Yakar, A; Ture, C; Saz, C (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 26: 18777-18789

Protection of water sources which are used for irrigation has raised great interest in the last years among the environmental strategists due to potential water scarcity worldwide. Excessive boron (B) in irrigation water poses crucial environmental problems in the agricultural zones and it leads to toxicity symptoms in crops, as well as human beings. In the present research, economic water treatment models consist of dried common wetland plants (*Lemna gibba, Phragmites australis*, and *Typha latifolia*) and *Lemna gibba* accumulation was tested and assessed to create a simple, cost-effective, and eco-friendly method for B removal from irrigation water. Significant amount of B was removed from irrigation water samples by EWTMs and B concentrations decreased below <1 mgL^{-1} when the components were exposed to 4 and 8 mgL^{-1} initial B concentrations. Moreover, the results from batch adsorption study demonstrated that dried *L. gibba* had a higher B loading capacity compare to other dried plants, and B sorption capacity of dried *L. gibba* was found as 2.23 mg/g. The optimum pH value for sorption modules was found as neutral pH (pH=7) in the batch adsorption experiment. Boron sorption from irrigation water samples fitted the Langmuir model, mostly B removed from irrigation water during the first 2h of contact time. Techno-economic analysis indicated that EWTM is a promising method that appears to be both economically and ecologically feasible, and it can also provide a sustainable and practical strategy for farmers to prevent B toxicity in their agricultural zones.

Impact of pH on the treatment of artificial textile wastewater containing azo dyes using pond systems

Yaseen, DA; Scholz, M (2019) INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH 13: 367-385

Two controlled experiments were operated to evaluate the impact of pH on the treatment efficiency of azo dyes within artificial textile wastewater using ponds as a polishing step. The objectives were to assess the (1) inflow water quality; (2) the presence of *Lemna minor* and the algae *Oedogonium* spp. on the dye removal and the outflow water quality; and (3) suitability of applying artificial textile wastewater and the impact of this wastewater and the dye on plants. Findings indicate that the shallow ponds planted with *L. minor* and/or algae treated the dye Basic Red 46 (BR46) significantly ($p<0.05$) higher than the control ponds without any significant ($p>0.05$) impact of pH. The potential of *L. minor* and algae for removal of BR46 was 31% and 25%, respectively. The removal of BR46 was around 89% for ponds planted with *L. minor* and algae, 58% for algae ponds and 33% for control ponds. Wetlands with *L. minor* are successful and economic in the complete degradation of BR46 at concentrations of 10mg L^{-1} for any pH value between 6 and 9. The removal of ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N) and ortho-phosphate-phosphorus (PO_4-P) was higher in planted than in unplanted ponds. The mean outflow values of chemical oxygen demand, NH_4-N, NO_3-N, suspended solids and total dissolved solids were within the limits for safe discharge. The artificial

wastewater constituents after dilution (1 part of artificial wastewater to 24 parts of raw water) were suitable to use within the treatment system containing *L. minor*. Article Highlights Planted ponds treat Basic Red 46 significantly better than other dyes and controls. No significant impact for systems at pH values of 6 and 9 regarding dye removal.Chemical oxygen demand and suspended/dissolved solids outflows were acceptable. Ammonium-nitrogen and nitrate-nitrogen outflows were below discharge thresholds. Diluted artificial wastewater and the presence dyes inhibited *Lemna minor* growth.

Uptake of some elements with aquatic plants exposed to the effluent of wastewater treatment plant

Tatar, S; Obek, E; Topal, EIA; Topal, M (2019) POLLUTION 5: 377-386

In this study, in the removal of macro (P, S, Na, K, Ca, Mg) and micro (Sb, Ba, Co, Cu, Fe, Pb, Mn, Hg, Mo, Se, Ag, Zn) elements in the effluent of Elazig Wastewater Treatment Plant, the efficiency of *Lemna minor* L. and *Lemna gibba* L. has been studied comparatively. For this aim, fronds of these plants have been adapted to the effluent of the treatment plant that feeds pilot scale reactors. The concentrations of elements in fronds harvested during the working period were analyzed by ICP/MS. The analytical results show that *Lemna minor* L. has a high accumulation potential for P, Sb, Ba, Co, Fe, Pb, Mn, Hg, Ag and Zn while *Lemna gibba* L. has a high accumulation potential for S, Na, Ca, Mg, Cu, Mo and Se. The results show that *Lemna minor* L. and *Lemna gibba* L. can be used as phytoremediators of wastewater.

Chromium and cadmium removal from wastewater using duckweed *Lemna gibba* L. and ultrastructural deformation due to metal toxicity

Chaudhary, E; Sharma, P (2019) INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 21: 279-286

Phytoremediation potential of *Lemna gibba* was evaluated for chromium (Cr) and cadmium (Cd) under laboratory conditions for variable metal load of 1 mg/l, 3 mg/l, 5mgl, 7 mg/l and 9 mg/l, respectively, for 7 and 15 days of treatment period. Effects of both metals on structural attributes of *L. gibba* were also analyzed by Scanning Electron Microscopic (SEM) study. The metal removal percentage by *L. gibba* for Cr metal was found in the range of 37.3% to 98.6% and for cadmium it was found within the range of 81.6% to 94.6%. Bio concentration factor (BCF) of *L .gibba* was observed within the range of 37 to 295 for Cr metal and for Cd metal it ranged from 237 to 1144, which shows that the plant is a hyper accumulator for Cd metal and moderate accumulator for Cr metal. Statistical analysis (Two-way ANOVA) was performed on experimental results to confirm the individual effect of metal concentration and treatment period as well as cumulative effect of both factors together on percentage metal removal and on BCF. Research studies indicated that with the progress of treatment period metal removal percentage increases but increasing metal load during experiment negatively co-relates the metal removal percentage and BCF.

Ammonia removal from chicken manure digestate through vapor pressure membrane contactor (VPMC) and phytoremediation

Ortakci, S; Yesil, H; Tugtas, AE (2019) WASTE MANAGEMENT 85: 186-194

Ammonia removal from synthetic ammonia solutions and chicken manure digestate via vapor pressure membrane contactor through Polytetrafluoroethylene (PTFE) membrane was investigated. The highest ammonia mass flux, separation factor, and removal efficiencies of 28.6 ± 0.2 g N/m^2 h, 53.9 ± 10.7, and 97.6 ± 0.7% were observed for synthetic solutions, respectively. Ammonia removal efficiency of 93.6 ± 1.9% through membrane contactor was observed for chicken manure digestate decreasing the total ammonia concentration from 3643.5 ± 67.2 to 230.9 ± 46.2 mg N/L. Phytoremediation via *Lemna minor* species was used as a polishing step to remove remaining ammonia from the membrane contactor effluent. Total ammonia concentration was then decreased below 2 mg N/L through evaporation, nitrification, and plant uptake processes occurring in the phytoremediation containers. This study reveals that ammonia can be successfully removed via VPMC and phytoremediation systems and the process is implementable as it can be coupled to anaerobic digestion processes to recover ammonia and to prevent ammonia inhibition.

Potentials of duckweed (*Lemna gibba*) for treatment of 1,4-dioxane containing wastewater using duckweed multi-ponds system

Osama, R; Ibrahim, MG; Fujiia, M; Tawfik, A (2019) TECHNOLOGIES AND MATERIALS FOR RENEWABLE ENERGY, ENVIRONMENT AND SUSTAINABILITY (TMREES) Edited by:Salame, CT; Aillerie, M; Papageorgas, P; Perilhon, C; Haider, A; Vokas, G; Shaban, A; Jabur, A. Vol 157: 676-682

Duckweed (*Lemna gibba*) was investigated for the treatment of wastewater containing 1,4-dioxane (e.g., polyester industry wastewater). Three continuous flow duckweed-pond systems (DWP), i.e., one pond, two ponds, and three ponds, were operated achieving different hydraulic retention time (HRT) of 2, 4 and 6 d, respectively. Results indicated that DWP3 is the most efficient one in 1,4-dioxane and NFL-N removals from wastewater. 1,4-dioxane and NFL-N removal efficiencies by DWP3 (56.9 ± 25% and 87.2 ± 7.1%, respectively) were slightly higher than that obtained by DWP2 (44.8 ± 19.6% and 81.9 ± 8.6%, respectively). It was found that, at DWP3, the pH value was reduced from 8.80 to 7.45, the dissolved oxygen (DO) was increased from 3.5 ± 1.9 to 7.5 ± 3 mg/L, and the concentration of total dissolved solids (TDS) was decreased from 921.5 ± 120.6 to 837.6 ± 83.6 mg/L. Eventually, duckweed (*Lemna gibba*) is effective to remove 1,4-dioxane from wastewater, representing an eco-friendly, effective and low operation and maintenance costs technology.

Effect of a mixture of cadmium and lead on nitrate and phosphate removal by the duckweed *Lemna gibba*

Aggoun, A; Benmaamar, Z (2019) ANNALI DI BOTANICA 9: 53-62

The purpose of the present study is to examine the effect of binary mixtures of the heavy metals, cadmium (Cd) and lead (Pb), on the assimilation of nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) from N and P-rich culture medium of the duckweed *Lemna gibba*. In binary mixtures, varied Cd concentrations (0.01, 0.1 and 1mg/L) were tested with 0.1 and 1 mg Pb/L respectively. Experiments were run for 2, 4, 6 and 8 days. Results showed that in all treatments (Cd+Pb), orthophosphates concentration decreased markedly within the two days of initiating experiments. The highest phosphate removal efficiency is 89.39%, in the solutions containing a binary mixture of Pb at 0.1 mg/L combined with Cd mg/L on the fourth day and about 83% in a binary mixture of Pb and Cd at 1mg/L each metal. Nevertheless, nitrate removal was weak with time. The co-contamination of the plant culture medium with varied Cd+Pb concentrations has no effect on phosphate removal, as

compared to the control. Additionally, Cd and Pb were removed from the medium and accumulated on *Lemna gibba*.

Analysis on enrichment of aquatic plants response to different heavy metal ions in polluted water taking duckweed as an example

Ouyang, Z; Yang, Z; Feng, G; Zhao, Y (2019) APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 17: 3469-3482

In order to improve the quality of water, the enrichment of different heavy metal ions by aquatic plants is studied taking duckweed as an example The site of the experiment is in Sichuan University of China, mainly from the following aspects of research: the heavy metal content in water, the heavy metal content in duckweed, chlorophyll content, adsorption capacity of cadmium ions in different concentrations of nitric acid solution, enrichment capacity of cadmium ion and the relationship between heavy metal content in duckweed and that of water. The study shows that the aquatic plants such as duckweed have a strong enrichment to Cu, Pb and Zn in the complex water body of heavy metals Cu, Pb and Zn, and their enrichment ability is higher than that of the water plants. With the increase of Cd^{2+} concentration, the content of chlorophyll a and b in duckweed decreased significantly, and the decline is increased with the increase of Cd^{2+} concentration. In 3 mg/L Cadmium nitrate solution, duckweed has a maximum absorption rate of Cd^{2+} reaching 87%, the adsorption of duckweed decreased with increasing concentration and the enrichment of Cd^{2+} in duckweed showed a nonmonotonic curve with the increase of treatment time. The concentration of Cd^{2+} in the duckweed of 8 d reached the maximum value (1.6 mg/g), and the concentration of Cd^{2+} is reduced in the 9-12 d duckweed, the concentration of heavy metals in water is significantly correlated with the concentration of Cu, Pb, Zn and Mn in aquatic plants duckweed. The method can effectively analyze the supposed enrichment. The sensitivity of duckweed can be used to detect changes of heavy metals in water, which can be used as an bioindicator of environmental pollution. At the same time, the accuracy of heavy metal determination by this method is high.

Phytoremediation as a tool for remediation of wastewater resulting from dyeing activities

Ugya, AY; Hua, X; Ma, J (2019) APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 17: 3723-3735

The production of dye is usually associated with several intermediate products and waste which are usually toxic and bio-hazardous and often disturb the environmental equilibrium. The present study is aimed at selecting macrophytes with the highest efficiency in the remediation of wastewater generated from dyeing activities. The test macrophytes were introduce into three different troughs for each plant containing 100% wastewater, 50% wastewater and control (100% borehole water). After 21 days the macrophytes were removed for further analysis. The reduction efficiency, Heavy metal bioconcentration and bio translocation factor of the macrophytes was determined. The result obtained shows that the test macrophytes improved the pH but were not able to improve the color of the wastewater. *Lemna minor* have the highest reduction efficiency for BOD, COD, Oil, and Grease while *Pistia stratiotes* have the highest reduction efficiency for TDS and TSS, *Eichhornia crassipes* have the highest reduction efficiency for Cl- and *Salvinia molesta* have the highest reduction efficiency for phenol and ammonical Nitrogen. All macrophytes recorded high reduction efficiency for heavy metal removal but *Pistia stratiotes* and *Eichhornia crassipes* had the highest

bioconcentration and translocation. These macrophytes should be introduced to the local dye users as a costless and environmentally friendly way of treating wastewater resulting from dyeing activity.

Integrated comparisons of thorium(IV) adsorption onto alkali-treated duckweed biomass and duckweed-derived hydrothermal and pyrolytic biochar

Chen, T; Zhang, N; Xu, Z; Hu, X; Ding, ZH (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 26: 2523-2530

In order to remove aqueous radionuclides and find an appropriate method for the disposal of wild duckweed in eutrophic water body, alkali-treated duckweed biomass and duckweed-based hydrothermal biochar (hydrochar) and pyrolytic biochars of 300 and 600°C were prepared. Their physicochemical properties were characterized carefully. The adsorption isothermal data fitted well with the Langmuir model and the maximum Langmuir adsorption capacities were 104.1, 96.3, 86.7, and 63.5mg/g for hydrochar, modified biomass, and 300 and 600°C biochars, respectively. The adsorption kinetic data fitted well with the pseudo-second-order kinetic equation. The sorption data of fixed-bed column also confirmed the high efficient removal of Th(IV) and fitted well with the Thomas model. The duckweed-based hydrothermal biochar is a low-cost adsorbent for Th(IV) removal, and it is also a resource utilization technology of the duckweed collected from eutrophic water body.

Using nano-attapulgite clay compounded hydrophilic urethane foams (AT/HUFs) as biofilm support enhances oil-refinery wastewater treatment in a biofilm membrane bioreactor

Jiang, YM; Khan, A; Huang, HY; Tian, YR; Yu, X; Xu, Q; Mou, LC; Lv, JG; Zhang, PY; Liu, P; Deng, L; Li, XK (2019) SCIENCE OF THE TOTAL ENVIRONMENT 646: 606-617

Petroleum refinery wastewater (PRW) treatments based on biofilm membrane bioreactor (BF-MBR) technology is an ideal approach and biofilm supporting material is a critical factor. In this study, BF-MBR with nanoattapulgite clay compounded hydrophilic urethane foams (AT/HUFs) as a biofilm support was used to treat PRW with a hydraulic retention time of 5 h. The removal rate of 500 mg/L chemical oxygen demand (COD), 15 mg/L NH_4^+ and 180 NTU of turbidity were 99.73%, 97.48% and 99.99%, which were 23%, 20%, and 6% higher than in the control bioreactor, respectively. These results were comparatively higher than that observed for the sequencing batch reactor (SBR). The death rate of the *Spirodela polyrhiza* (L.) irrigated with BF-MBR-treated water was 4.44%, which was similar to that of the plants irrigated with tap water (3.33%) and SBR-treated water (5.56%), but significantly lower than that irrigated with raw water (84.44%). The counts demonstrated by qPCR for total bacteria, denitrifiers, nitrite oxidizing bacteria, ammonia oxidizing bacteria, and ammonia oxidizing archaea were also higher in BF-MBR than those obtained by SBR. Moreover, the results of 16 s rRNA sequencing have demonstrated that the wastewater remediation microbes were enriched in AT/HUFs, e.g., *Acidovorax* can degrade polycyclic aromatic hydrocarbons, and *Sulfuritalea* is an efficient nitrite degrader. In summary, BF-MBR using AT/ HUF as a biofilm support improves microbiome of the activated sludge and is reliable for oil-refinery wastewater treatment.

The treatment of duckweed with a plant biostimulant or a safener improves the plant capacity to clean water polluted by terbuthylazine

Panfili, I; Bartucca, ML; Del Buono, D (2019) SCIENCE OF THE TOTAL ENVIRONMENT 646: 832-840

Water pollution is becoming alarming since thousands contaminants are dispersed in the aquatic environments, and agricultural practices, for the massive use of pesticides, are contributing to exacerbating this problem. In this context, a research aimed at investigating the ability of duckweed (*Lemna minor*), a free-floating aquatic species widespread throughout the world, to remediate water polluted with five different concentrations of a herbicide - terbuthylazine (TBA) - was carried out. In addition, duckweed was treated with a plant biostimulant and a safener with the aim of increasing the plant's capacity to tolerate and remove the TBA from the water. The results evidenced that the herbicide affected the duckweed already at the lower concentrations, reducing its capacity to proliferate and the area of its fronds. On the contrary, when the TBA treatments were performed in combination with the biostimulant or the safener the average area of the fronds was affected of lesser extents, compared to the plants treated with the herbicide only. Antioxidant enzymes, namely ascorbate peroxidases (APX) and catalases (CAT) were investigated and it was found that the biostimulated and safened duckweed showed increased activities of these enzymes, compared to the plants treated with TBA only. At last, some phytofiltration experiments were planned. The biostimulated and safened duckweed removed more TBA from polluted water than the plants treated with the herbicide alone. In conclusion, this research showed that duckweed is suitable for cleaning water polluted with TBA and this potential can be successfully improved by treating the species with a biostimulant or a safener.

Integrating laboratory and field studies to assess impacts of discharge from a uranium mine and validate a water quality guideline value for magnesium

Trenfield, MA; Harford, AJ; Mooney, T; Ellis, M; Humphrey, C; van Dam, RA (2019) INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT 15: 64-76

Magnesium (Mg) is a primary contaminant in mine water discharges from the Ranger Uranium Mine (north Australia). Site-specific water quality guideline values (WQGVs) for Mg have been derived from laboratory and field studies. Contaminated groundwater with elevated electrical conductivity and metals (Mg, Mn, U, SO_4, and Ca) was detected flowing from the mine site into adjacent surface waters. This provided an opportunity to investigate the protectiveness of the Mg WQGV by conducting an integrated laboratory and field study. A direct toxicity assessment (DTA) of the groundwater was conducted with local tropical freshwater species: duckweed (*Lemna aequinoctialis*), green hydra (*Hydra viridissima*), and the aquatic snail *Amerianna cumingi*. An in situ toxicity assessment was carried out in the creek receiving diluted groundwater by use of the same species of snail, to aid interpretation of laboratory-derived data. The toxicity of the contaminated groundwater was higher than Mg-only toxicity testing for *H. viridissima*, with other elevated metals and major ions contributing to toxicity. However, for duckweed and snail, the contaminated groundwater was less toxic than the Mg-only testing. In situ snail monitoring supported laboratory exposures, showing no effect on reproduction of A. cumingi exposed to an average of approximately 5 mg/L Mg; however, a very small effect was noted closer to the groundwater source, probably associated with other contaminants. The minimal toxicity observed *for L. aequinoctialis* and *A.*

cumingi, despite the elevated Mg, can be explained by the high calcium (Ca) concentration of the water and the potential amelioration of metal toxicity. The extent of Ca amelioration of Mg toxicity was organism dependent. This study affirms the proposed environmental rehabilitation standard of 3 mg/L Mg for surface waters with a Ca concentration typical of water from this mine site.

Phytotoxicity

Evaluation of cobalt hyperaccumulation and tolerance potential of the duckweed (*Lemna minor* L.)

Hu, D; Cheng, MH; Hu, KJ; Zhang, W; Yang, YR; Xu, QS (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 179: 79-87.

Lemna minor could tolerate and accumulate more than 5,000 μg g^{-1} DW of cobalt (Co) without foliar symptoms, indicating it is a Co hyperaccumulator. However, the physiological and metabolomics mechanisms that are responsible for Co accumulation and tolerance are largely unknown. In the present study, Fourier transform infrared spectroscopy suggested that C=O, C-H, and O-H groups are involved in Co biosorption. The activation of antioxidant enzymes, such as superoxide dismutase, guiacol peroxidase, catalase, and glutathione reductase, as well as ascorbic acid and glutathione might be involved in capturing reactive oxygen species as evidenced by decreased malondialdehyde in fronds treated with Co. Metabolomics analysis revealed that Co stress significantly increased the production of several amino acids (except aspartic acid and cysteine at 200 μM) and organic acids (with the exception of succinic acid). In particular, an approximate 15-fold increase was noted in the citric acid concentration. Upon exposure to Co, increases were observed in citrate synthase, malate dehydrogenase, and phosphoenolpyruvate carboxylase activities, and a decrease was observed in isocitrate dehydrogenase related to the metabolism of organic acids. Overall, the increase in concentration of organic and amino acids and antioxidants support their effective involvement in improving Co tolerance and accumulation in *L. minor*.

Phytotoxicity and degradation of antibiotic ofloxacin in duckweed (*Spirodela polyrhiza*) system

Singh, V; Pandey, B; Suthar, S (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 179: 88-95

The phytotoxicity and degradation of ofloxacin (OFX) in duckweed *Spirodela polyrhiza* based system was estimated in this study. For that, OFX was added in an environmentally relevant range (0.01-1.0 mg L^{-1}) in medium (Hoagland nutrient) and toxicity biomarkers, i.e. changes in plant biomass, relative growth rate (RGR), photopigment (Chl-a, Chl-b and carotenoids), protein content, antioxidative enzymes (catalase, CAT; superoxide dismutase, SOD; and ascorbate peroxidases, APX) in fronds were estimated. The batch-scale setups (250 ml) was prepared in triplicate for each concentration of OFX and reared in growth chambers (Algae Tron AG 230) for 7 d. Results suggested that the high concentrations of OFX caused a reduction in biomass (4.8-41.3%), relative root growth (RGR), protein (4.16-11.28%) and photopigment contents. The fronds in OFX spiked setups showed an increased level of antioxidative enzymes: CAT (0.230-0.338 mmol(H_2O_2) mg^{-1} protein), APX (0.043-0.074 mmol(ascorbate) mg^{-1} protein), and SOD (0.267-0.317 U mg^{-1} protein) than control. At the end (7 d), the residual OFX content in the medium was also estimated, and results suggested a significant ($p < 0.05$) reduction (93.73-98.36%) in OFX content than control setup (54.76-75.53%) at the end of the

experimentation. The trend of residual OFX suggested phytodegradation as a significant mechanism of antibiotic degradation other than hydrolysis and photodegradation processes. This study indicates that duckweed can be an effective bio-tool for the removal of environmental relevant concentration of the antibiotics from the wastewater.

Modes of action and adverse effects of gamma radiation in an aquatic macrophyte *Lemna minor*

Xie, L; Solhaug, KA; Song, Y; Brede, DA; Lind, OC; Salbu, B; Tollefsen, KE (2019) SCIENCE OF THE TOTAL ENVIRONMENT 680: 23-34

High dose rates of ionizing radiation have been reported to cause adverse effects such as reduction in reproduction and growth, and damage to protein and lipids in primary producers. However, the relevant effects of ionizing radiation are still poorly understood in aquatic plants. This study was intended to characterize the biological effects and modes of action (MoAs) of ionizing radiation using gamma radiation as the prototypical stressor and duckweed *Lemna minor* as a model organism. *Lemna minor* was exposed to 1, 14, 24, 46, 70 mGy/h gamma radiation dose rates from a cobalt-60 source for 7 days following the testing principles of the OECD test guideline 221. A suite of bioassays was applied to assess the biological effects of gamma radiation at multiple levels of biological organization, including detection of reactive oxygen species (ROS), oxidative stress responses (total glutathione, tGSH; lipid peroxidation, LPO). DNA damage, mitochondrial dysfunctions (mitochondria) membrane potential, MMP), photosynthetic parameters (chlorophyll a, chl a; chlorophyll b, chl b; carotenoids; Photosystem II (PSII) performance; CO_2 uptake), intercellular signaling (Ca^{2+} release) and growth. Gamma radiation increased DNA damage, tGSH level and Ca^{2+} content together with reduction in chlorophyll content, maximal PSII efficiency and CO_2 uptake at dose rates between 1 and 14 mGy/h, whereas increases in cellular ROS and LPO, inhibition of MMP and growth were observed at higher dose rates (≥ 24 mGy/h). A network of toxicity pathways was proposed to portray the causal relationships between gamma radiation-induced physiological responses and adverse outcomes to support the development of Adverse Outcome Pathways (AOPs) for ionizing radiation-mediated effects in primary producers.

Toxicity of nanosilver and fumonisin B-1 and their interactions on duckweed (*Lemna minor* L.)

Radic, S; Domijan, AM; Ljubimir, KG; Maldini, K; Ivesic, M; Stefanic, PP; Krivohlavek, A (2019) CHEMOSPHERE 229: 86-93

In the environment co-contamination of several toxicants commonly occurs. However, toxicological studies usually are focused on only one toxicant. The aim of this study was to investigate toxicity of silver nanoparticles (AgNP) and mycotoxin fumonisin B^{-1} (FB1) and their possible interactions as well as to explore tentative mechanism of their toxic effect. Duckweed (*Lemna minor* L.) was treated with AgNP or FB1 (at concentrations 0.5 and 1.0 mg L^{-1}) or with their combination at same concentrations for 3 days. Both AgNP and FB1 applied individually significantly affected levels of certain nutrients, reduced growth rate and the levels of photosynthetic pigments though AgNP at a much greater extent compared to FB1. Furthermore, AgNP induced ROS generation, lipid peroxidation and increase of antioxidative enzymes activities, while FB1 induced changes only in the activities of antioxidative enzymes. Those results implicate that phytotoxicity of both AgNP and FB1 can be associated with imbalance of mineral and cell redox status. However, toxic actions of AgNp

singly applied were more pronounced. Combined treatment with AgNP and FB1 produced higher degree of changes in all parameters than corresponding concentrations of AgNP or FB1 alone implying their additive effects. Additionally, higher level of FB1 found in medium, and higher level of intracellular Ag following combined treatment indicates interaction of two toxicants at the transport level/uptake in the cell which resulted in higher accumulation of Ag in duckweed cells. The latter in turn exerted higher toxicity to duckweed compared to single treatment of AgNP.

Toxicity of tailing leachates from a niobium mine toward three aquatic organisms

Paquet, N; Indiketi, N; Dalencourt, C; Lariviere, D; Roberge, S; Gruyer, N; Triffault-Bouchet, G; Fortin, C (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 176: 355-363

The aim of this research was to assess the ecotoxicity of leachates originating from a niobium mine located in Canada. These tailings contain considerable amounts of carbonates and phosphates and could potentially be used as fertilizer for agriculture. However, the presence of different contaminants linked with the ores mined, including rare earth elements and daughter elements of the uranium disintegration chain is of concern. Bioassays have been used to determine if the tailings leachates could be harmful. The assessment of the toxicity of progressive dilutions of five tailing leachates (808, 809, 810, 811 and 897) was performed on different organisms: phytoplankton *Raphidocelis subcapitata* and duckweed *Lemna minor*, based on their growth and chlorophyll a content, and water flea *Daphnia magna* based on their mobility, mortality and reproduction. Overall, the leachates showed higher toxicity to *Raphidocelis subcapitata* and *Lemna minor*, than toward *Daphnia magna*. Leachate 808 showed no toxicity to all organisms while leachate 810 showed significant effects to all species. The results can be explained by the leachate dissolved metal or nutrient concentrations, but also by the metal bioavailability which depends on pH and hardness. Generally, toxicity was observed in undiluted samples tested, which is not representative of the conditions that could occur in the environment. This supports the idea that these tailings could be used as fertilizer albeit more studies may be required, particularly to assess the toxicity of the tailings leachate for benthic organisms, the toxicity of the tailings for terrestrial organisms and the variations of soil and sediment physicochemical properties after tailing treatments.

Stability and toxicity studies for duloxetine and econazole on *Spirodela polyrhiza* using chiral capillary electrophoresis

Valimana-Traverso, J.; Amariei, G.; Boltes, K.; Garcia, M.A.; Marina, M.L. (2019) JOURNAL OF HAZARDOUS MATERIALS 374:203-210

Stability and toxicity studies for duloxetine and econazole were achieved using individual solutions and their mixtures. Stability of drugs racemates and enantiomers was investigated under abiotic and biotic conditions. Toxicity was evaluated for the first time on *Spirodela polyrhiza*. EC_{50} values were calculated for each individual drug and for their binary mixture. Real (not nominal) concentrations determined by Capillary Electrophoresis were employed in the calculations of toxicity parameters. The use of a 25mM phosphate buffer (pH 3.0) with 1.5% S-beta-CD as chiral selector at a temperature of 30°C and a separation voltage of -20kV enabled the simultaneous enantiomeric separation of duloxetine and econazole in 7.5min with enantiomeric resolutions of 7.9 and 6.5, respectively. For individual solutions, decay percentages under abiotic conditions were higher for duloxetine (80%) than for econazole (60%), while in presence of *Spirodela polyrhiza* they increased

for duloxetine but not for econazole. Econazole showed the highest decay percentages under abiotic or biotic conditions (100%) in binary mixtures. EC_{50} values for duloxetine and econazole enabled to include both drugs within the group of very toxic compounds although econazole showed a higher toxicity than duloxetine and the binary mixture.

Effects of copper ions on removal of nutrients from swine wastewater and on release of dissolved organic matter in duckweed systems

Zhou, Q; Li, X; Lin, Y; Yang, CP; Tang, WC; Wu, SH; Li, DH; Lou, W (2019) WATER RESEARCH 158: 171-181

High concentration of Cu^{2+} in swine wastewater raises concerns about its potential adverse effects on nutrient removal by aquatic plants like duckweed. In this work, the effects of copper ions on nutrient removal and release of dissolved organic matter (DOM) were investigated in duckweed systems. Results showed that the removal performance of ammonia nitrogen (NH_3-N) and total phosphorus (TP) increased at 0.1-1.0 mg/L of Cu^{2+}, while dropped at 2.0-5.0 mg/L of Cu^{2+}. A novel kinetic model in which Cu^{2+} was taken into account was then developed which was used to optimize Cu^{2+} concentration at 0.96 mg/L for nutrient removal in duckweed systems. NADH, detected in DOM by the parallel factor (PARAFAC) analysis, exhibited high capacities of binding copper ions, so it played an important role on the decrease of Cu^{2+} concentrations in duckweed systems. The principle component analysis (PCA) showed that the dominant DOM were lower molecular weight compounds at 1.0 mg/L of Cu^{2+} and higher molecular weight compounds at 2.0-5.0 mg/L of Cu^{2+}. The bonds of C-H (humic-like), N=0 (NO_3^-) and Ar-H (tyrosine) in DOM were responsible for not only the fastest binding with Cu^{2+} from the result of the two-dimensional Fourier transform infrared correlation spectroscopy (2D-FTIR-CoS) but also the variations of DOM conformations at a critical concentration of 0.5 mg/L Cu^{2+} from the perturbation correlation moving window two-dimensional (PCMW2D) analysis. These findings lead to a better understanding on the environmental behaviours and mechanisms of Cu^{2+} in duckweed systems.

Radiation effects and ecological processes in a freshwater microcosm

Hevroy, TH; Golz, AL; Hansen, EL; Xie, L; Bradshaw, C (2019) JOURNAL OF ENVIRONMENTAL RADIOACTIVITY 203: 71-83

Ecosystem response to gamma radiation exposure depends on the different species sensitivities and the multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels. In this study, multi-species microcosms were used to investigate effects of ionizing radiation in a model freshwater ecosystem, including endpoints at both structural and functional levels and ecological interactions. Microcosms were exposed for 22 days to a gradient of gamma radiation with four dose rates from 0.72 to 19 mGy h^{-1}, which are within the range of those seen at contaminated sites. Results showed significant dose related effects on photosynthetic parameters for all macrophyte species. No significant effects of radiation were observed for the consumers in the microcosms, however trends indicate the potential for longer-term effects. We also witnessed a different response of *Daphnia magna* and *Lemna minor* compared to previous single-species studies, illustrating the importance of multispecies studies, which aim to encompass systems more realistic to natural ecosystems. Microcosms allowed us to isolate specific relationships between interacting species in an ecosystem and test the effects, both direct and indirect, of radiation on them. In addition, the

ecological pathways and processes, and the experimental design itself, was central to understanding the results we witnessed. This type of study is important for radioecology research that has been very much limited to high dose rates and single species studies. This approach to radioecology has been strongly promoted in recent decades and, to our knowledge, this is the first microcosm study performed at dose rates similar to those at contaminated field sites.

Uranium biosorption by *Lemna* sp. and *Pistia stratiotes*

Vieira, LC; de Araujo, LG; Ferreira, RVD; da Silva, EA; Canevesi, RLS; Marumo, JT (2019) JOURNAL OF ENVIRONMENTAL RADIOACTIVITY 203: 179-186

Biosorption-based technologies have been proposed for the removal of radionuclides from radioactive liquid waste containing organic compounds. Nevertheless, pytoremediation potential of uranium (U) by non-living aquatic macrophytes *Lemna* sp. and *Pistia stratiotes* has not been previously addressed. In this study, uranium biosorption capacity by *Pistia stratiotes* and *Lemna* sp. was evaluated by equilibrium and kinetics experiments. The biomasses were added to synthetic and real waste solutions. The assays were tested in polypropylene vials containing 10 mL of uranium nitrate solution and 0.20 g of biomass. Solutions ranging from 0.25 to 84.03 mM were employed for the assessment of uranium concentration in each macrophyte. The equilibrium time was 1 h for both macrophytes. *Lemna* sp. achieved the highest sorption capacity with the use of the synthetic solution, which was 0.68 mmol g^{-1} for the macrophyte. Since *Lemna* sp. exhibit a much higher adsorption capacity, only this biomass was exposed to the actual waste solution, being able to adsorb 9.24 x 10^{-3} mmol g^{-1} U (total). The results show that these materials are potentially applicable to the treatment of liquid radioactive waste.

Iron oxide nanoparticle phytotoxicity to the aquatic plant *Lemna minor*: effect on reactive oxygen species (ROS) production and chlorophyll a/chlorophyll b ratio

Souza, Lilian Rodrigues Rosa; Bernardes, Luis Eduardo; Barbetta, Maike Felipe Santos; da Veiga, Marcia Andreia Mesquita Silva (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH INTERNATIONAL DOI:10.1007/s11356-019-05713-x

Although iron oxide occurs naturally in the environment, iron oxide nanoparticles have distinct mobility, reactivity, and toxicity, which can harm the human health and nature. This scenario has motivated the investigation of the toxic effects of iron oxide nanoparticles (akaganeite predominance + hematite) on the aquatic plant *Lemna minor*. First, nanoparticles were synthesized and characterized; then, different iron oxide NP concentrations were added to *Lemna minor* culture. After 7days, all the *Lemna minor* leaves died, irrespective of the added NP concentration. The iron oxide NP impact on the plant was evaluated based on malondialdehyde (MDA) production from thiobarbituric acid reactive substances (TBARS), which was dose-dependent; i.e., lipid peroxidation in the plant increased with rising iron oxide NP concentration. The chlorophyll content decreased at high iron oxide NP concentrations, which disrupted the light absorption mechanism. Fe accumulation in Lemna minor roots also occurred, which can harm nutrient uptake. Therefore, the iron oxide NP toxic impact on plants and related ecosystems requires further studies in order to prevent environmental damage.

Comparative ecotoxicity of single and binary mixtures exposures of nickel and zinc on growth and biomarkers of *Lemna gibba*

Martinez, R S; Saenz, M E; Alberdi, J L; Di Marzio, W D (2019) ECOTOXICOLOGY DOI:10.1007/s10646-019-02065-7

The aim of this study was to compare the ecotoxicity of nickel (Ni) and zinc (Zn) assayed as single and as binary mixture. In addition, how were affected the population growth rates and oxidative stress biomarkers, comparing single to binary exposures. The toxicity tests were performed on *Lemna gibba* using a 7-day test. All calculations were made using measured total dissolved metal concentrations. IC_{50}-7d, based on growth rate calculated on frond number and fresh weight, were 2.47/3.89mg/L, and 76.73/76.93mg/L, for Ni and Zn, respectively. Single metals affected plant growth following a non-linear concentration-response relationship. LOEC values for each metal were obtained at 0.92 and 20.1mg/L for Ni and Zn, respectively. Biomarkers of the antioxidant response like Catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APOX; EC 1.11.1.11) and guiacol peroxidase (GPOX; EC 1.11.1.7) activities in single metals assays were higher than controls, but when similar concentrations were added as mixtures, that increase was reduced and inhibition with respect to the control was observed for GPOX. APOX showed the highest activity. The concentration addition (CA) approach was evaluated and resulted in a correct predictor of Ni-Zn mixture toxicity on Lemna gibba. This was made comparing the EC_{50} and LOEC, measured taking the growth rate as endpoint, with those expected values according to the CA model. However, the measured biomarkers indicating a positive response to free radicals did not fit to concentration addition model when assayed in the binary mixture. Also, the main activity response of these was observed within a range of concentrations below the LOEC values for the mixture.

The impact of humic acid on toxicity of individual herbicides and their mixtures to aquatic macrophytes

Mihajlovic, Varja; Tomic, Tanja; Tubic, Aleksandra; Molnar Jazic, Jelena; Ivancev Tumbas, Ivana; Sunjka, Dragana; Lazic, Sanja; Teodorovic, Ivana (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH INTERNATIONAL DOI:10.1007/s11356-019-05629-6

This study investigates the impact of humic acid (HA) on the toxicity of selected herbicides and their binary mixtures to aquatic plants. The focus was on two auxin simulators (2,4-D and dicamba) and two photosynthetic inhibitors (atrazine and isoproturon). The results suggested that the addition of HA to the standard synthetic medium does not affect Lemna minor growth nor the toxicity of atrazine, but increases the toxicity of 2,4-D and the binary mixture of atrazine and 2,4-D. The addition of HA to the standard synthetic medium reversibly decreased the growth (biomass) of Myriophyllum aquaticum and enhanced the toxicity of individually tested herbicides (isoproturon and dicamba) as well as their binary mixture. The results showed delayed toxic effects of auxin simulators, especially 2,4-D in the Lemna test. The recovery after the exposure to individual photosystem II inhibitors (atrazine and isoproturon) is fast in both plant species, regardless of the presence of HA. In the case of selected mixtures (atrazine + 2,4-D and isoproturon + dicamba), recovery of both plant species was noted, while the efficiency depended on the herbicide concentration in the mixture rather than the presence or absence of HA.

Orthophosphate modulates the phytotoxicity of nano-ZnO to *Lemna minor* (L.)

Chen, X; O'Halloran, J; Jansen, MAK (2019) ENVIRONMENTAL TECHNOLOGY 40:2446-2454

Because of their applications in large numbers of products, Zinc Oxide nanoparticles (nano-ZnO) will inevitably enter into the environment. Nano-ZnO released into the environment will be present in a complex matrix which can cause various chemical and physical transformations and modulate the biological reactivity of these particles. Due to their rapid growth and small size, *Lemna minor* is recommended by OECD for toxicological testing. Here, we tested how nano-ZnO reactivity is modulated by the suite of macro- and micronutrients that are present in *Lemna minor* growth media. Specifically, we measured ex situ Reactive Oxygen Species (ROS) formation by nano-ZnO, and subsequent in planta toxicity. The data show how orthophosphate can modulate both ex situ ROS formation, and in planta toxicity. This has ramifications for phytotoxicity testing, which is commonly performed under controlled conditions and on media containing orthophosphate.

Phytotoxicity assessment of isoproturon on growth and physiology of non-targeted aquatic plant *Lemna minor* L. - A comparison of continuous and pulsed exposure with equivalent time-averaged concentrations

Varga, M; Horvatic, J; Zurga, P; Brusic, I; Moslavac, M (2019) AQUATIC TOXICOLOGY 213:105225

Phenylurea herbicides are often present in the aquatic ecosystems and may be accumulated by the non-targeted organisms and impose a negative effect on the organism and the community. This study aims to investigate and compare the effects of two different isoproturon (IPU) pulse exposure scenarios on the non-targeted aquatic plant *Lemna minor* with effects observed in the standard test with continuous exposure. The obtained results showed that continuous IPU treatment causes significant reduction of photosynthetic pigment concentration and proteins as well as inhibition of *L. minor* growth. The activities of CAT, G-POX, and APX were significantly induced to diminish the accumulation of ROS under IPU treatment, but the induction of antioxidant enzymes was not sufficient to protect the plants from herbicide-induced oxidative stress. The growth of *L. minor* under pulse exposure to IPU recovers fast, but pulse treatment results in significant physiological changes in treated plants. The accumulation of H_2O_2 and lipid peroxidation products, alongside the reduced concentration of proteins and photosynthetic pigments in pulse treatment after a recovery period, indicates that IPU causes prolonged oxidative stress in *L. minor* plants. The recovery potential of *L. minor* plants after treatment with herbicides may have an important role in maintaining the population of essential primary producers in aquatic ecosystems, but IPU-induced physiological changes could potentially have a significant role in modulating the response of the plants to the next exposure event.

Growth, physiological function, and antioxidant defense system responses of *Lemna minor* L. to decabromodiphenyl ether (BDE-209) induced phytotoxicity

Sun, Y; Sun, P; Wang, CT; Liao, JH; Ni, JP; Zhang, TAN; Wang, RS; Ruan, HH (2019) PLANT PHYSIOLOGY AND BIOCHEMISTRY 139: 113-120

Polybrominated diphenyl ethers (PBDEs), represent one of the new types of persistent organic pollutants (POPs) that are currently found in ambient aquatic ecosystems. *Lemna minor* L. is a floating freshwater plant, which is widely employed for phytotoxicity studies of xenobiotic substances. For this study, we investigated the growth, physiological functions, and antioxidant capacities of *L. minor*, which were exposed to 0-20 mg L^{-1} decabromodiphenyl ether (BDE-209) for 14 days. A logistic model was suitable for describing the growth of *L. minor* when the BDE-209 concentration was in the range of from 0 to 15 mg L^{-1}. When exposed to 5 and 10 mg L^{-1} BDE-209, the growth of *L. minor* was significantly increased, where the intrinsic rate (r) and the maximum capacity of the environment (K) of *L. minor* were significantly higher than those of the control. In this case, the chlorophyll content and soluble proteins were also markedly increased. Moreover, the photosynthetic function (Fv/Fm, PI) was enhanced. However, for 15 mg L^{-1} BDE-29 treated group, the growth of *L. minor* was significantly inhibited, with decreases in chlorophyll and the soluble protein content, until the *L. minor* yellowed and expired under a concentration of 20 mg L^{-1}. Photosynthetic functions were also negatively correlated with increasing increments of BDE-209 (15 and 20 mg L^{-1}). The malondialdehyde (MDA), superoxide anion radical (O^{-}_{2}(-center dot)) content, and permeability of the plasma membranes increased with higher BDE-209 concentrations (0-20 mg L^{-1}). The superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities of *L. minor* increased when the BDE-209 concentration ranged from 0 to 10 mg L^{-1}; however, the activities of SOD and POD were decreased. Only the CAT activity remained higher in contrast to the control group under 15-20 mg L^{-1} BDE-209. These results demonstrated that 15 mg L^{-1} BDE-209 imparted high toxicity to *L. minor*, which was a consequence of the overproduction of reactive oxygen species (ROS), which conveyed oxidative damage to plant cells.

Does intraspecific variability matter in ecological risk assessment? Investigation of genotypic variations in three macrophyte species exposed to copper

Dumont, ER; Larue, C; Lorber, S; Gryta, H; Billoir, E; Gross, EM; Elger, A (2019)m AQUATIC TOXICOLOGY 211: 29-37

To limit anthropogenic impact on ecosystems, regulations have been implemented along with global awareness that human activities are harmful to the environment. Ecological risk assessment (ERA) is the main procedure which allows to assess potential impacts of stressors on the environment as a result of human activities. ERA is typically implemented through different steps of laboratory testing. The approaches taken for ERA evolve along with scientific knowledge, to improve predictions on ecological risks for ecosystems. We here address the importance of intraspecific variability as a potential source of error in the laboratory evaluation of pollutants. To answer this question, three aquatic macrophyte species with different life-history traits but with their leaves directly in contact with the water were chosen; *Lemna minor* and *Myriophyllum spicatum*, two OECD model species, and *Ceratophyllum demersum*. For each species, three or four genotypes were exposed to 7-8 copper concentrations (up to 1.9 mg/L, 2 mg/L or 36 mg/L for *C. demersum*, *L. minor* and *M. spicatum*, respectively). To assess species sensitivity, growth-related endpoints such as Relative Growth Rate (RGR), based either on biomass production or on length/frond production, and chlorophyll fluorescence F-v/F-m, were measured. For each endpoint, the effective concentration 50% (EC_{50}) was calculated. Almost all endpoints were affected by Cu exposure, except F_v/F_m, of *M. spicatum*, and resulted in significant differences among genotypes for Cu sensitivity. Genotypes of *L. minor* exhibited up to 35% of variation in EC_{50} values based on F_v/F_m, showing differential sensivity among

genotypes. Significant differences in EC_{50} values were found for RGR based on length for *M. spicatum*, with up to 72% of variation. Finally, *C. demersum* demonstrated significant sensitivity differences among genotypes with up to 78% variation for EC_{50} based on length. Overall, interspecific variation was higher than intraspecific variation, and explained 77% of the variation found among genotypes for RGR based on biomass, and 99% of the variation found for F_v/F_m. Our results highlight that depending on the endpoint, sensitivity can vary greatly within a species, and that pollutant- and species-specific endpoints should be considered in ERA.

The effects of photon-upconversion nanoparticles on the growth of radish and duckweed: Bioaccumulation, imaging, and spectroscopic studies

Modlitbova, P; Hlavacek, A; Svestkova, T; Porizka, P; Simonikova, L Novotny, K; Kaiser, J (2019) CHEMOSPHERE 225: 723-734

In this study, radish (*Raphanus sativus* L) and common duckweed (*Lemna minor* L.) were treated with an aqueous dispersion of carboxylated silica-coated photon-upconversion nanoparticles containing rare-earth elements (Y, Yb, and Er). The total concentration of rare earths and their bioaccumulation factors were determined in root, hypocotyl, and leaves of *R. sarivus* after 72 h, and in *L. minor* fronds after 168 h. In *R. sativus*, translocation factors were determined as the ratio of rare earths concentration in hypocotyl versus root and in leaves versus hypocotyl. The lengths of the root and hypocotyl in *R. sativus*, as well as the frond area in *L. minor*, were monitored as toxicity endpoints. To distinguish rare earth bioaccumulation patterns, two-dimensional maps of elemental distribution in the whole *R. sativus* plant and *L. minor* fronds were obtained by laser-induced breakdown spectroscopy with a lateral resolution of 100 μm. Moreover, the bioaccumulation was inspected using a photon-upconversion laser microscanner. The results revealed that the tested nanoparticles became adsorbed onto *L. minor* fronds and *R. sativus* roots, as well as transferred from roots through the hypocotyl and into leaves of *R. sativus*. The bioaccumulation patterns and spatial distribution of rare earths in nanoparticle-treated plants therefore differed from those of the positive control. Overall, carboxylated silica-coated photon-upconversion nanoparticles are stable, can easily translocate from roots to leaves, and are expected to become adsorbed onto the plant surface. They are also significantly toxic to the tested plants at nominal concentrations of 100 and 1000 μg/mL.

The biological activities of prothioconazole enantiomers and their toxicity assessment on aquatic organisms

Zhai, WJ; Zhang, LL; Cui, JN; Wei, YM; Wang, P; Liu, DH; Zhou, ZL (2019) CHIRALITY 31: 468-475

Chiral fungicide prothioconazole has a wide range of antifungal spectrum; however, little research has been conducted to evaluate prothioconazole on an enantiomeric level. Five target pathogens and three common aquatic organisms were tested for the enantioselective bioactivity and toxicity of prothioconazole in this work. The antifungal activity of the enantiomers against wheat phytoalexin, rice blast fungus, *exserohilum turcicum*, *Alternaria triticina*, and *Fusarium avenaceum* was determined, and it was found that (-)-prothioconazole were 85 to 2768 times more active than (+)-prothioconazole toward these target organisms. In order to reflect the risk to aquatic ecosystem, the acute toxicity of the enantiomers to *Daphnia magna*, *Chlorella pyrenoidosa*, and *Lemna minor* L. was assessed. It was observed that the toxicity of (-)-prothioconazole to *D. magna* was 2.2 times higher

than (+)-prothioconazole, but it was lower to *C. pyrenoidosa* and *L. minor* L. The toxicities of (+)-enantiomer and (-)-enantiomer to *D. magna* and *C. pyrenoidosa* were synergy, indicating that the racemate had higher threat to the organisms. It could be concluded that the effects of prothioconazole on target organisms and the acute toxicity to nontarget species were enantioselective with (-)-enantiomer possessing higher efficiency and lower toxicity. Such enantiomeric differences should be taken into consideration when assessing the performance of prothioconazole.

Diclofenac as an environmental threat: Impact on the photosynthetic processes of *Lemna minor* chloroplasts

Hajkova, M; Kurnmerova, M; Zezulka, S ; Babula, P; Vaczi, P (2019) CHEMOSPHERE 224: 892-899

Mechanisms of pharmaceuticals action on biochemical and physiological processes in plants that determine plant growth and development are still mostly unknown. This study deals with the effects of non-steroidal anti-inflammatory drug diclofenac (DCF) on photosynthesis as an essential anabolic process. Changes in primary and secondary photosynthetic processes were assessed in chloroplasts isolated from *Lemna minor* exposed to 1, 10, 100, and 1000 μM DCF. Decreases in the potential and effective quantum yields of photosystem II (F_V/F_M by 21%, Phi(II) by 44% compared to control), changes in non-photochemical fluorescence quenching (NPOJ, and a substantial drop in Hill reaction activity (by 73%), especially under 1000 μM DCF, were found. Limitation of electron transport through photosystem II was confirmed by increased fluorescence signals in steps) and I (by 50% and 23%, respectively, under 1000 μM DCF) in OJIP fluorescence transient. Photosystem I exhibited changes only in the redox state of P700 reaction centres (decrease in P_m by 10%, increase in reduced P_{700} by 5% under 1000 μM DCF). Similarly, RuBisCO activity was only lowered by 30% under 1000 μM DCF. In contrast, a significant increase in reactive oxygen and nitrogen species (by 116% and 157%, respectively) was observed under 10 μM DCF, and lipid peroxidation increased even at 1 μM DCF (by nearly seven times compared to the control). Results demonstrate the ability of environmentally relevant DCF concentrations to induce oxidative stress in isolated duckweed chloroplasts; however, photosynthetic processes were affected considerably only by the highest DCF treatments.

Study of the accumulation of contaminants by *Cyperus alternifolius*, *Lemna minor*, *Eichhornia crassipes*, and *Canna generalis* in some contaminated aquatic environments

Shirinpur-Valadi, A; Hatamzadeh, A; Sedaghathoor, S (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH DOI:10.1007/s11356-019-05203-0

Today, environmental pollution, especially heavy metal pollution, is known as a new and possibly more dangerous pollutant than other environmental ones. For this purpose, the uptake of four aquatic plants in different environments was chosen. In this experiment, four macrophytes, i.e., umbrella palm (*Cyperus alternifolius*), duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), and canna (*Canna generalis*), were studied in five contaminated aquatic environments, i.e., Gohar Rood river, Zarjoob river, Eynak lagoon, Anzali lagoon, and control solution (containing Cd, Cr, Pb, and Zn). The results showed that the highest uptake rates of cadmium, cobalt, vanadium, chromium, zinc, nickel, and lead were observed for duckweed fronds. The highest bioconcentration factor (BCF) of nickel was related to duckweed stem and water hyacinth root, and the highest BCF of

cadmium belonged to duckweed fronds and canna root. The highest rate of uptake of cadmium, chromium, zinc, and lead was related to control. The least amount of uptake of several metals by plants was obtained from the water of Gohar Rood and Zarjoob. Generally, based on the results of this study, it can be stated that duckweed is suitable for the uptake of most heavy metals.

Ecotoxicity risk of presence of two cytostatic drugs: Bleomycin and vincristine and their binary mixture in aquatic environment

Jureczko, M; Przystas, W (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 172: 210-215

Cytostatic drugs have become one of the greatest environmental threats. They occur in surface, ground and even drinking water. Their key emission sources are hospital effluents, municipal wastewater, as well as drug manufacturers and their effluents. These compounds are extremely stable in natural waters and they are not significantly removed during wastewater treatment, because they are resistant to biodegradation. The aim of this work was to establish possible negative effects of chosen cytostatics: bleomycin and vincristine on the three trophic levels of surface waters. A single agent acute toxicity test was conducted on representatives of the producer an aquatic freshwater plant *Lemna minor*, the consumer - crustaceans *Daphnia magna*, and the decomposer - bacteria *Pseudomonas putida*. Binary mixture tests were performed according to the Concentration Addition, Response Additivity, and Independent Action models. Both substances had a different effect on the tested organisms; bleomycin could be classified as a very toxic, while vincristine as a toxic water pollutant. Half maximal effective concentration (EC_{50}) values designed in the presented single agent acute toxicity studies are < 10 mg/L in all the tests with bleomycin as well as vincristine conducted on *L. minor*. In tests with vincristine performed on *D. magna* and *P. putida* EC_{50} > 100 mg/L. The highest toxicity is demonstrated by bleomycin towards the aquatic freshwater plant (EC_{50} = 0.2 mg/L). The binary mixture of the tested chemicals showed antagonistic effects of environmental concern.

The first comprehensive safety study of Magneli phase titanium suboxides reveals no acute environmental hazard

Kokalj, AJ; Novak, S; Talaber, I; Kononenko, V; Mali, LB; Vodovnik, M; Zegura, B; Elersek, T; Kalcikova, G; Gotvajn, AZ; Kralj, S; Makovec, D; Caloudova, H; Drobne, D (2019) ENVIRONMENTAL SCIENCE-NANO 6: 1131-1139

Magneli phase titanium suboxides (Magneli TiO_x) are novel conductive materials with great industrial potential. This is the first comprehensive investigation to provide an extensive dataset for hazard characterisation of Magneli TiO_x using six standardised test organisms and two human cell lines. Three Magneli TiO_x particles and three anatase crystalline phase TiO2 nanomaterials with different primary powder sizes, but similar hydrodynamic diameters in biological media, were investigated. All Magneli TiO_x and TiO_2 nanomaterials induced negligible biological responses in *Tetrahymena thermophila*, *Lemna minor*, *Artemia franciscana* and *Danio rerio*. Adverse effects were found only at 100 mg L^{-1} where approximately 50% endpoint inhibition was recorded in the cases of *Daphnia magna* exposed to two Magneli TiO_x, *Pseudokirchneriella subcapitata* exposed to all three Magneli TiO_x and HepG2 cells exposed to one type of Magneli TiO_x. This study provides the first evidence that the tested Magneli TiO_x are not acutely hazardous according to existing EU classification for conventional chemicals.

Toxic effects of Pb on *Spirodela polyrhiza* (L.): Subcellular distribution, chemical forms, morphological and physiological disorders

Sha, Sha; Cheng, Menghua; Hu, Kaijie; Zhang, Wei; Yang, Yaru; Xu, Qinsong (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 181:146-154

The impact of lead (Pb) on *Spirodela polyrhiza* was studied to determine the subcellular distribution, chemical forms, and resulting morphophysiological modifications after treatments with 20 or 80 μM $Pb(NO_3)2$ for 10 days. At the subcellular level, the Pb uptake by *S. polyrhiza* was mainly compartmentalized in the cell walls (70%), and the majority of Pb (approximately 70%) was extracted using 1 M NaCl and 2% acetic acid (HAc). Visual symptoms of phytotoxcity, surface roughness and closure of stomata, were observed in Pb-treated fronds. Electron-dense precipitates were present in cell walls, and changes to the ultrastructure were most noticeably exhibited in organelle shape, internal organization, and size of the plastoglobules of chloroplasts. Toxic concentrations of Pb induced oxidative stress in fronds, characterized by an accumulation of malondialdehyde (MDA) and decreased chlorophyll and unsaturated fatty acid contents. Pb exposure increased ABS/RC, TRo/RC, DIo/RC, Vj, and phiDo (F_v/F_m), indicating that reaction centers were transformed to dissipation sinks, leading to a decrease in the efficiency of photosystem II, which was evident from the decreased values of F_v/F_o, F_v/F_m, psiEo, phiEo, RC/ABS, and PI_{abs}. These results indicated that decreased photosynthesis in Pb-treated fronds was partially ascribed to the lower pigment content, inhibition of electron transport, inactivation of the reaction centers, damage to the chloroplast ultrastructure, and stomatal closure. The physiological implications of subcellular distribution and chemical forms are discussed in relation to Pb accumulation and detoxification. However, Pb accumulation significantly impaired photosynthesis and membrane integrity in the fronds of *S. polyrhiza*.

Herbicides in the cave environment: ecotoxicological risks

Havelkova, B; Hlavkova, D; Kovacova, V; Beklova, M (2019) FRESENIUS ENVIRONMENTAL BULLETIN 28: 781-786

This study aimed to detect triazine pesticides and their metabolites in the drip water of the Amaterska cave system (Moravian karst, Czech Republic). Ecotoxicological tests were used to assess the risk of selected herbicides (i.e. atrazine and terbuthylazine) and their metabolites (atrazinedesethyl, atrazine-desisopropyl, terbuthylazinedesethyl) in the drip water by means of bioassays with selected bioindicators. Tests were conducted on organisms representing all trophic levels of the aquatic ecosystem: producer algae *Pseudokirchneriella subcapitata* and macrophyte duckweed *Lemna minor*, a consumer water flea *Daphnia magna*, and decomposer bacterium *Vibrio fischeri*. Effects of herbicides on edaphon representatives were measured by testing the reproductive inhibition of enchytraeids *Enchytraeus crypticus*. Triazines and their metabolites were analysed in the drip water of the Amaterska cave system sampled at a site under agricultural cropland. Standard methods were used for testing procedures: OECD 201 (Freshwater algal growth inhibition test), OECD 221 (*Lemna* sp. Growth Inhibition Test), OECD 202 (Inhibition of the mobility of *Daphnia magna*), ISO 11348-2 (Inhibitory effect of water samples on the light emission of *Vibrio fischeri*) and OECD 220 (*Enchytraeid* Reproduction Test). Drip water samples were examined for 350 substances. Six pesticides above the limit of quantification were identified; all of these substances belong to the triazine pesticide group. The performed experiments showed no evidence of acute toxicity of the drip water. However, accumulation in the environment and chronic toxicity could not be ruled out. The

ecotoxicity of selected herbicides and their metabolites differed significantly depending on the test organism and testing conditions.

Evaluation of platinum nanoparticles ecotoxicity using representatives of distinct trophic levels of aquatic biocenosis

Hlavkova, D; Beklova, M; Kopel, P; Havelkova, B (2019) NEURO ENDOCRINOLOGY LETTER 39:465-472

The aim of this study was to clarify the influence of three different sizes of platinum nanoparticles on aquatic ecosystem and assess the toxic effect in term of particle size. Tests were conducted on organisms representing all trophic levels of the aquatic ecosystem, namely producers (duckweed *Lemna minor*), consumers (water fleas *Daphnia magna*) and decomposers (bacteria *Vibrio fischeri*). Experiments were carried out methodologically in accordance with the following standards: OECD 221 guideline (*Lemna* sp. Growth Inhibition test), OECD 202 guideline (Inhibition of the mobility of *Daphnia magna*) and ISO 11348-2 (Inhibitory effect of platinum nanoparticles on the light emission of *Vibrio fischeri*). The most toxic have been the smallest sized platinum nanoparticles for all tested organisms. The highest toxicity of all tested samples (Pt1, Pt2, Pt3) was observed in bacteria (30 EC_{50} = 135.47; 167.94; 254.64 $g.L^{-1}$), respectively. The lowest toxicity was recorded for *Daphnia* (48h EC_{50} = 405.74; 413.24; 514.07$g.L^{-1}$), respectively. The ecotoxicity of platinum nanoparticles varies considerably according to the test organisms and particle size.

Freshwater chronic ammonia toxicity: A tropical-to-temperate comparison

Mooney, TJ; Pease, CJ; Hogan, AC; Trenfield, M; Kleinhenz, LS; Humphrey, C; van Dam, RA; Harford, AJ (2019) ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 38: 177-189

The chronic toxicity of ammonia to tropical freshwater species is understudied, and thus data on temperate species have been used to derive water quality guideline values for tropical regions. Such practices may lead to underprotective guideline values due to differences in toxicities observed between tropical and temperate species. In addition, the presence of ammonia in low-ionic-strength waters may also result in higher toxicity, and studies on this factor are limited. The present study assessed the toxicity of ammonia to 6 tropical freshwater species in low-ionic-strength waters. Because ammonia toxicity varies depending on the pH and temperature, test water pH concentrations were maintained at approximately pH 6.0 ± 0.3 at temperatures between 27.5 and 30°C. Low-effect chronic inhibition concentrations were derived for the following species: *Chlorella* sp. 66 mg L^{-1}; *Lemna aequinoctialis* 22 mg L^{-1}; *Hydra viridissima* 1.8 mg L^{-1}; *Moinodaphnia macleayi* 27 mg L-*1*; *Amerianna cumingi* 17 mg L-*1*; and *Mogurnda mogurnda* 5.4 mg L^{-1} total ammonia nitrogen. Two of the species tested (a cnidarian and a fish species) were among the most sensitive reported anywhere within their taxonomic group. Chronic ammonia datasets representing toxicity estimates for temperate and tropical species were plotted and compared using species sensitivity distributions. The results indicate that the differences in chronic toxicity observed between tropical and temperate species were likely due to the low ionic strength of the waters to which tropical species were exposed, rather than any inherent physiological differences between species from tropical and temperate regions. This finding suggests that tropical waters of low ionic strength may be at a higher risk from ammonia compared with other freshwater ecosystems.

Unusual Applications/Studies

Lemna gibba and *Eichhornia crassipes* extracts: Clean alternatives for de-acidification, antioxidation and fungicidal treatment of historical paper

Mohamed, WA; Mansour, MMA; Salem, MZM (2019) JOURNAL OF CLEANER PRODUCTION 219: 846-855

In this research *Lemna gibba* (L) and *Eichhornia crassipes* (E) ethanolic extracts were added to pure cellulose substrate to produce impregnated interleaving papers (LIP) and (EIP). These products are proposed to cure historical paper undergoing acidification, oxidation and fungal infection. The extracts were analysed using Gas Chromatography/Mass Spectrometer (GC/MS) and polyphenolic compounds were assessed. The products were tested on model decayed paper showing identical characteristics of historical paper, on which pH values were measured, transition metals concentrations were evaluated using Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) and samples were investigated using Scanning Electron Microscope combined with Energy Dispersive Spectroscopy (SEM-EDX) respectively. Radical scavenging and antioxidation activities of the extracts were also assessed using 1,1-diphenyl-2-picryl-hydrazyl free radical DPPH. Results demonstrated a significant increase ($P < 0.01$) in the DPPH. Free radical scavenging with increasing concentration in the following order vitamin C > BHT approximate to (L) extract > (E) extract. Results showed also that both (LIP) and (EIP) products effectively neutralized the acidity of decayed paper after 7 day treatment and evidently chelated transition metals. Zn^{2+} and Fe^{2+} were better chelated by (LIP) treatment in the order; $Zn^{2+} > Fe^{2+} > Pb^{2+} > Cu^{2+}$, while Pb^{2+} and Cu^{2+} were greater removed by (EIP) treatment in the order $Pb^{2+} > Cu^{2+} > Zn^{2+} > Fe^{2+}$. Up to 90% radical scavenging was achieved at 320.00 mu g/mL concentration of (L) extract and 667.296 mu g/mL concentration of (E) extracts. *Aspergillus niger*, *Penicillium roqueforti* and *Eurotium chevalieri* growth are inhibited at 1000 µg/mL concentration of LIP with considerable fungal spots elimination. Our findings established LIP and EIP products as novel natural origin alternative for effective synergic deacidification, antioxidation, antiradical, metal chelation and fungicidal treatment of historical paper.

Effects of homeopathic preparations of mercurius corrosivus on the growth rate of severely mercury-stressed duckweed *Lemna gibba* L.

Jager, T; Wurtenberger, S; Baumgartner, S (2019) HOMEOPATHY: THE JOURNAL OF THE FACULTY OF HOMEOPATHY 108: 128-138

We developed a bioassay with mercury-stressed duckweed (*Lemna gibba* L.) to study potential effects of homeopathically potentised mercury(II) chloride (Mercurius corrosivus [Merc-c.]). The response of this bioassay to homeopathic treatments as a function of stress intensity was also of interest. Duckweed was severely stressed with mercury(II) chloride for 48 hours. Afterwards plants grew in either Merc-c. (seven different potency levels, 24x to 30x) or water controls (unsuccussed and succussed water) for 7 days. Growth rates of the frond (leaf) area were determined using a computerised image analysis system for different time intervals between the measurements on days 0, 3 and 7. Three independent experiments with potentised Merc-c. each were evaluated. Additionally, three water control experiments were analysed to investigate the stability of the experimental set-up (systematic negative control [SNC] experiments). All experiments were

randomised and blinded. Unsuccussed and succussed water did not significantly differ in terms of duckweed growth rate. The SNC experiments did not yield any significant effects, providing evidence for the stability of the experimental system. Data from the two control groups and the seven treatment groups (Merc-c. 24x-30x) were each pooled to increase the statistical power. Duckweed growth rates for day 0 to 3 were reduced ($p<0.05$) after application of Merc-c. compared with the controls. Growth rates for day 3 to 7 were not influenced by the homeopathic preparations. The present test system with *Lemna gibba* L. that was severely stressed by mercury yielded evidence for specific effects of Merc-c. 24x to 30x, namely a growth reduction in the first time period (day 0-3). This is in contrast to former experiments with slightly arsenic-stressed duckweed, where a growth increase was observed in the second time period (day 2-6). We hypothesise that the differing results are associated with the level of stress intensity (severe versus slight).

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

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Copyright and co-author consent

All listed authors must concur in the submission and the final version must be seen and approved by all authors of the contribution. As a public forum, we do not carry out any Copyright application. If you need to copyright your material, please do so beforehand.

Formatting requirements:

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- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
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Title:

- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
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    - be introduced in their full form (e.g., Visualization of Polarized Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) Activity in Live Cells by Fluorescence Resonance Energy Transfer (FRET) Imaging); or
    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

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Links for Further Reading

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

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

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

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

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