

# DUCKWEED FORUM



**ISCDRA**  
International Steering Committee on  
Duckweed Research and Applications

Volume 5 (1), issue 16, pages 1 - 31 (2017)



*Landoltia punctata* 7260



*Lemna perpusilla* 8473



*Wolffia neotropica* 7279



*Wolffia lingulata* 8237

*Landoltia punctata* is a common species found in many continents and locations. It was variously named as *Spirodela oligorrhiza*, *Spirodela punctata* and *Spirodela sichuanensis* until molecular taxonomic studies at and around 1999 revealed that it is likely a new genus and thus renamed in honor of the late-Elias Landolt. The clone 7260 is currently under cytogenetic investigations in the lab of Ingo Schubert (IPK, Gatersleben, Germany) using probes derived from the *S. polyrrhiza* 7498 genome project. *Lemna perpusilla* is a species that is endemic to eastern and central North America. A famous clone 6746 was originally designated as *L. perpusilla* and was chosen as a model system for seminal work on flowering induction in duckweed by William Hillman and Herbert Posner around the 1970's. However, it has been reclassified as *Lemna aequinoctialis* and reported by Landolt in 1986. *Wolffiella lingulata* is a species found in warmer regions of the America continent. This species shows an interesting saddle-shaped frond with the tip bent downward into the water and its base on the water surface. On solid media, we noted that this species often generates circular colonies with whirls of fronds stacked on each other. Another species found only in the warmer regions of South America is *Wolffiella neotropica*. This species has very similar morphologies to those of *W. lingulata* except for having more stomata (35 vs. 10) per frond and lower frequency of flowering. Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

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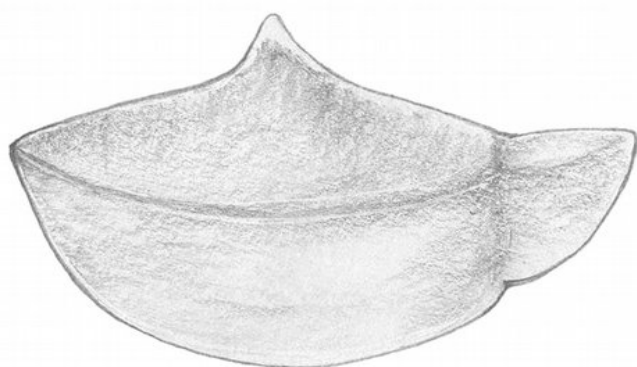
## International Steering Committee on Duckweed Research and Applications Members

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Information about the ISCDRA: <http://lemnapedia.org/wiki/ISCDRA>

All prior Duckweed Forum issues: [http://lemnapedia.org/wiki/Duckweed\\_Forum](http://lemnapedia.org/wiki/Duckweed_Forum)

## Science meets art: *Wolffia brasiliensis*



0.5 mm

*Wolffia brasiliensis* Wedd. can be found in the tropical and subtropical eastern regions of the American continents. The fronds are broad, ovate having a prominent papilla in the middle of the dorsal surface. It is therefore commonly called “papillate watermeal”, which might hint towards its applications. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

# Letter from the editor

Dear friends of duckweed research and applications,

When you open this file with the new issue of our "Duckweed Forum" then we are facing the New Year 2017. We wish you all a prosperous, successful year of 2017- not only but also with duckweed research or applications. This is Issue number 16 of the "Duckweed Forum", starting Volume 5.

First of all we want to focus your attention on our upcoming **4th International Conference on Duckweed Research And Applications** (ICDRA-2017), which will be held at the Central University of Kerala in Periyar, Kerala, India. The Chair of the conference, Dr. K. Sowjanya Sree, Periyar and co-Chair, Prof. Dr. Jitendraa Khurana of Delhi, invite you to attend this important meeting and provide information on traveling options for reaching this venue. If you have any questions, please do not hesitate to contact Sowjanya at the addresses given. We hope to see you all at this meeting.

During the General Assembly of the 4<sup>th</sup> ICDRA, the newly elected Steering Committee will take over the responsibility from the present one. We ask you to suggest candidates for the committee. The conditions are explained. Also we announced the request for applications to host the next conference in 2019. We will look forward to see your application for it.

There is more in store when you read the newsletter. Simona Paolacci from the University College of Cork, Ireland is introduced at "Student Spotlight". Two reports from Thailand tell you interesting details about using duckweed for human nutrition and how to prepare your own duckweed dish. Several high school students from Germany have prepared and tasted selected meals enriched with duckweed. They gave their opinion and ratings in their report. I hope you will find these articles of interest as well as tempting your culinary interests.

There was also an account on the first "International Duckweed Industry Workshop" in Argentina that was organized by the ILA. Read what one participant, Paul Fourounjian from Rutgers University NJ, wrote about it.

In addition, from our community is a first response on how to fight algae in duckweed cultures. This common problem remains to be discussed more by duckweed researchers and developers. Our "Discussion Corner" is open for your comments and I look forward to hearing about your experience and contributions.

We summarized again the newest duckweed literature ("From the database"), Dr. K. Sowjanya Sree presents a drawing of *Wolffia brasiliensis* under "Science meets Art" and the cover page is designed with photos of duckweed species by Prof. Dr. Eric Lam, Rutgers University NJ. Unfortunately, in DF number 14, two errors sneaked in. Please, read our "Corrigendum" in this issue. In the internet version for this issue of DF, both errors should be corrected now.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),

Klaus-J. Appenroth, Chair



# ICDRA-2017 Conference information: 23-26 October, 2017

## How to Reach the Venue: Central University of Kerala, Periyar, India

Our greetings to you all for the ongoing festive season and we extend our welcome once again to the 4th International Conference on Duckweed Research and Applications to be held at the Central University of Kerala, India from 23- 26 October, 2017.

Central University of Kerala is located at Tejaswini Hills, Periyar in the northern district called Kasaragod of Kerala state. Kerala is one of the southernmost states of India, longitudinally spanning the southwest coast line as well a considerable part of one of the three biodiversity hotspots of India, the Western Ghats.

How to reach the venue?

**By air:** The closest airport is situated at Mangalore, Karnataka, India. It is ~80 km north of Periyar, Kerala (Venue). The Bajpe airport at Mangalore is an international airport having direct flights to Dubai, Abu Dhabi and Sharjah. One would find plenty of options for connecting flights to other parts of the world via these air hubs. Mangalore is also well-connected to other international airports in India: Mumbai, Bangalore, Hyderabad and Delhi.

The ~80 km stretch from airport to the venue, along the west coast, can be covered either by road or by railway.

**By road:** A taxi from Mangalore International Airport to Periyar charges Rs. 2,500.00 (current fares) for a two to two and half hours ride depending on the traffic flow.

For someone traveling from inland, direct buses are available from different parts of Kerala. The University main gate is conveniently located on the National Highway with a bus stop for all local and express bus services passing through.

**By train:** The closest railway station is situated 12 km away at Kanhangad, which receives trains from various parts of the country. More information on train timing and availability may be obtained from [www.indianrail.gov.in](http://www.indianrail.gov.in) and [www.irctc.co.in](http://www.irctc.co.in) . Kanhangad is well connected by bus lines to the Central University campus at Periyar.

**If you have any additional questions** or you need an invitation right now, please contact Dr. Sowjanya Sree at her email address.

Once again, we wish you all a Merry Christmas and a very Happy New Year!

Co-Chair of the Conference

**Prof. Dr. Jitendra P. Khurana**

Department of Plant Molecular Biology

University of Delhi South Campus

New Delhi, India

Chair of the Conference

**Dr. K. Sowjanya Sree**

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# Announcing Call for ISCDRA Nominees

## **Order for the election of the members of the International Steering Committee on Duckweed Research and Applications**

The International Steering Committee on Duckweed Research and Applications (ISCDRA) was founded during the 2nd International Conference on Duckweed Research and Applications (ICDRA) at Rutgers, the State University of New Jersey, New Brunswick, NJ in 2013.

Members of the committee cooperate with each other in order to steer and promote duckweed research and applications for the benefit of our community. Publishing the ISCDRA Duckweed Forum is one of the obligations, among others, that is expected of the committee members.

- 1) The ISCDRA should consist of 5 members who will be elected before the biannual ICDRA in a secret poll.
- 2) Anyone who has previously attended any of the ICDRA or will be attending it this year, or receives the ISCDRA Newsletter can suggest potential candidates including themselves up to 6 weeks before the meeting. Candidates should have attended any of the previous 3 ICDRA meetings. Suggestions may be sent to the present head of the ISCDRA- Dr. Klaus J. Appenroth, Email: [election@lemnapedia.org](mailto:election@lemnapedia.org) up to 6 weeks before the conference: this will be 11<sup>th</sup> of September 2017.
- 3) The voting procedure will be announced in the next Duckweed Forum issue.
- 4) The five newly elected members will be notified by email and they will elect the head of the committee before the ICDRA.
- 5) In case that by chance all elected members are either from the applied field or from the research field, the elected Chair will appoint one additional member from the missing field.
- 6) At the end of the ISCDRA meeting (General Assembly) the previous Committee reports shortly about the activities since the previous election and the duty is transferred to the newly elected ISCDRA.



# Request for Applications to Host ICDRA- 2019

In order to identify the best venue possible for the next meeting of the ICDRA, applications from interested organizations are requested to be sent to the ISCDRA:

[steering-committee@lemnopedia.org](mailto:steering-committee@lemnopedia.org).

The applications should briefly introduce the proposed venue, its benefit/attractions, relevance to duckweed research and/or applications, and the responsible organizer's credentials as well as experience. The list of all suggestions will be send out with the last "Duckweed Forum" before the ICDRA and decided during the "General Assembly" at the end of the conference on the 23rd – 26th October 2017, in Central University of Kerala, Periyar.

# Student Spotlight: Ms. Simona Paolacci

Email: [spaolacci@ucc.ie](mailto:spaolacci@ucc.ie)

I graduated in Environmental Science at the Sapienza University of Rome. Following my graduation I worked for an environmental association and for a waste management company. In 2010, I completed a Masters degrees with a two-year research program in Environmental Monitoring and Restoration. My research project focused on botany applied to phytoremediation. I tested the ability of genetically modified *Nicotiana tabacum* plants to produce adventitious roots. In 2009, I founded, along with other young biologists, a company that managed many activities, such as environmental monitoring and environmental education, inside a protected area near Rome.

I liked my job but I missed research and I wanted to experience another country- working on my PhD thesis research abroad was my dream. I had no contact with foreign universities and I wanted to improve my English, so, in March 2011, I left Italy to go to Kinsale, County Cork in Ireland to work as an *au pair*. After 6 months I moved to Cork City. I finally felt confident with my English and I knew that I was ready to go back into science.

I visited the University College of Cork website where I found the profile of Prof Marcel Jansen. His research on plant eco-physiology appealed to me. I did not hesitate to contact him. Marcel introduced me to duckweed research, and especially the question of why the invasive American species *Lemna minuta* was spreading in Ireland, and displacing native *Lemna minor*. Marcel proposed to me an internship and I accepted the offer with enthusiasm. In November 2011, with Marcel's help, I wrote a proposal for a PhD-project. In April 2012 the Irish Research Council communicated to me that my funding application was successful. In the following October, I started my PhD in the School of Biological, Earth and Environmental Sciences. (BEES)

For my PhD thesis research, I investigated the performance of *Lemna minuta* (invasive in Europe) under different environmental conditions and compared it with the performance of the co-generic native *Lemna minor*. The aim of my study was to identify environmental factors that explain the success of one species over the other. I used physiological and morphological parameters to quantify the performance of the two species and interpret the growth strategies adopted. I grew the two species using different nutrient concentrations, different light intensities and in the presence of several physical and chemical stressors.

In parallel, for two years, I monitored the presence and abundance of *L. minuta*, and *L. minor* in natural freshwater ponds where the two species co-occur. The field monitoring included water analysis and assessment of other environmental parameters. I also carried out a year-long field experiment in order to investigate the performance of *L. minuta* and *L. minor* throughout the seasons. As a final stage I collected clones of *L. minuta* and *L. minor* in different areas of Ireland and, with the help of Prof Appenroth (University of Jena, Germany) and Dr Bog (University of Regensburg, Germany), I genotyped my clones using AFLP and DNA sequencing.



In November 2016 I finished my PhD and, at the moment, I am working as a senior demonstrator and research assistant at BEES. In July 2015, I attended the third International Conference on Duckweed Research and Application at Kyoto, Japan. This conference revealed to me the fantastic opportunities to utilize Lemnaceae for addressing environmental issues. I realized that I would really enjoy studying duckweeds from this new perspective. In particular, I am interested in the use of duckweed for wastewater treatment and protein production.



I have just submitted a proposal for a postdoctoral fellowship focusing on the development, in Ireland, of an integrated system that will include the use of *Lemnaceae* species to treat fish farm wastewater and the extraction of valuable proteins from the biomass produced. During my PhD I acquired a deep knowledge of duckweed physiology and gained experience in culturing these plants both in laboratory as well as under field conditions. It is now my wish to use my expertise that I have gained from my postgraduate training to address environmental and economic problems that affect our society.





# Corrigendum

Recently, it came to our attention that there are two errors in the published Duckweed Forum (DF), Issue 14, which we need to correct. On the cover page, the species names on the two lower pictures were inadvertently interchanged. Thus, *Wolffia brasiliensis* 8116 should be on the left panel while *W. australiana* 8730 should be on the right panel. On the Science meets art section, the word "*Wolffiella*" was misspelled as "*Wolfiella*".

The corrected version for this Issue of DF is already in place of the old version at the Lemnapedia ([http://lemnapedia.org/wiki/Duckweed\\_Forum#2016-07](http://lemnapedia.org/wiki/Duckweed_Forum#2016-07)) as well as the RDSC ([http://www.rduckweed.org/uploads/1/0/8/9/10896289/iscdra-duckweedforum\\_issue14-2016-07-2.pdf](http://www.rduckweed.org/uploads/1/0/8/9/10896289/iscdra-duckweedforum_issue14-2016-07-2.pdf)).

## Indigenous Cuisine with a Modern Flare

**Dr. Wanpen Saengthongpinit** Division of Food Science and Technology, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Nakhon Pathom 73000, Thailand.

Known around the world as water-meal, *Wolffia globosa* is called “phum”, “kai phum”, “kai nhum”, or “kai nahe” by the Thai people. Water-meal is eaten by Thais and Thai-Lao descendants especially in the northern and northeastern regions of Thailand. Used in various local dishes such as red curry and stir fry, water-meal has high nutritional value (94.8% moisture, 1.33% protein, 0.66% fiber, 0.1% fat, 1.48% ash, 422 µg/g d.b. beta-carotene, 49.6 mg GAE/g d.b. total phenolics)<sup>1</sup> and high antioxidant activities (98.4 % inhibition in DPPH assay, 99.3 % inhibition in ABTS assay)<sup>1</sup>. Even though it can be found in markets around Thailand, water-meal is not yet considered a common food among the other ethnic communities.



Some people have reported dislike of water-meal due to its odor and sandiness to the palate. To overcome these problems, we have initiated a research program to develop more contemporary menus that incorporate 2-70% water-meal and conducted sensory evaluation. Among the preferred main dishes were steamed egg, omelet, fish ball green curry, fried egg in sour soup, fried fish curry, and baked spinach with cheese. Popular appetizers included fried sandwich, fried bread with minced pork spread, steamed rice noodle roll, and sandwich spread. Favorite desserts were green tea ice cream, green tea cookies, banana cake, and doughnut. We hope that our efforts will attract a wider range of Thai consumers to this ‘kept-secret’ indigenous food source.

<sup>1</sup>Composition and properties may depend on growth conditions (reference: Saengthongpinit, W., Sricharoen, B. and M. Krangpreecha (2016) “Drying water-meal (*Wolffia globosa*) and using in food products for Lao Khrang, Huay Duan Sub-district, Dontoom District, Nakhon Pathom Province, Nakhon Pathom”, Research report (in Thai) to Nakhon Pathom Rajabhat University).



# *Lemna minor* – alternative food for vegetarians and non-vegetarians?

**Celine Sendelbeck, Sheila Zoepel, Justin Boerngen Roman-Herzog-Gymnasium Schmoelln, Thuringia, Germany**

During our 10th school class we are required to prepare “Seminarfacharbeit” – a thesis on the basis of experimental work. In December 2015 we decided to select duckweed as an experimental plant and to cultivate it in a 25 L aquarium. We received for our experiments *Lemna minor*, clone 9441 (Kleine Wasserlinse, Common Duckweed), isolated many years back in Germany. It was not easy to get the cultivation of duckweed started; they grew hardly at all in the beginning. We changed temperature as well as the light regime. Luckily, after some weeks we decided to use the commercially available fertilizers “Easy-Life ProFito” and “EasyCarbo”

<http://www.aquaristikshop.com/aquaristic/Easy-Life-ProFito/305120/> and [http://www.zooplus.co.uk/shop/fish/aquatic\\_plants\\_care/algae\\_products/51139](http://www.zooplus.co.uk/shop/fish/aquatic_plants_care/algae_products/51139).

Thereafter, during 3 months (February 2016 to May 2016), we produced sufficient biomass to ask one vegetarian and one non-vegetarian (one of the authors, SZ) to test self-made meals (prepared by one of the authors, CS) containing duckweed. Each small meal (see below) of approximately 60 g contained between 5 and 13 g of fresh *Lemna minor*. The tasting experiments were carried out for 20 days. The results were presented below.

We carried out also a small survey and got the feedback that presently most people have some reservation to accept

duckweed as human food. Many of them, however, are ready to test them by curiosity. A nutritionist confirmed that eating duckweed is healthy and no health risk can be expected.



From left: Sheila Zoepel, Celine Sendelbeck and Justin Boerngen.



	bad not recommended	neutral no specific taste	good	super	
<b>Non-vegetarian food</b>	Fish and potato		<input type="radio"/> unusual, but not bad		
	Loin (pork)			✓	
	Instant mashed potato (fast food)		<input type="radio"/> odd		
	Pasta stew	✗			
	Pasta with tomato sauce				✓ top
	Potato salad			✓	
	Bread, butter and salami				✓
	Cutlet and potato			✓	
	Rice stew: good			✓	
	Bread, butter and bacon			✓	
	Doner		<input type="radio"/> not so good	✓	
	Bean stew			✓	
	Scrambled eggs			✓	
	Fish finger, potato			✓	
	Chicken, mashed potato			✓	
<b>Vegetarian based food</b>	Bread and cream cheese		<input type="radio"/>		
	Bread and yellow cheese		<input type="radio"/>		
	Bun with herb butter		<input type="radio"/>		
	Omelette with cheese		<input type="radio"/>		
	Scrambled eggs			✓ partially like cress	
	Asparagus and potato			✓	
	Pasta with pesto			✓	
	Bread with avocado			✓	
	Baked potato, curd cheese			✓	
	Bread with yellow cheese			✓	
	Bread and butter			✓	
	Lettuce, carrots, cucumber			✓ very good	
	Lettuce, tomato, garden radish			✓ very good	

# Wolffia Spicy Salad - a recipe to share

Recipe by Orathai Pakdee

## Ingredients

- 1. ½ kg fresh *Wolffia*
- 2. 1 cup of sliced tomatoes
- 3. 1 cup of sliced onion
- 4. ½ cup of cut Chinese celery
- 5. 5 spoons of lime juice
- 6. 1 spoon of fish sauce
- 7. ½ spoon of sugar
- 8. ¼ cup of chopped chili pepper

## Directions

1. Wash *Wolffia* thoroughly several times until the rinsed water becomes clear.

2. Boil *Wolffia* in boiling water for 2 min. Drain the water using a fine mesh sieve or cheesecloth, and let it stand to cool down.

3. Prepare the dressing by mixing lime juice, fish sauce and sugar in a separate bowl. Adjust the taste to your liking (Note: Thai people usually like it sour and slightly sweet).

4. Add the dressing, tomatoes, onion, Chinese celery and chili pepper (you can reduce the amount to lower spiciness) to the *Wolffia* in a large bowl. Mix well.

Note: It can be served with warm white rice, or spread over a biscuit for European flavor.





# Discussion Corner

## Re: “Natural Approaches to Algae Treatment in Duckweed Production” Article from Duckweed Forum #15

Reader response: I am a PhD student in Dr. Eric Lam's lab at Rutgers University. I work on duckweed and I just read your discussion article in the Duckweed Forum about treatment of algae in duckweed production ponds. I have come across this problem as well and have tried increasing duckweed surface area and reducing light exposure like you mentioned. However, I have found that decreasing the pH significantly reduces the amount of algal growth. I grow my duckweed on Hoagland's media, not in open ponds, but I found that the optimal pH is 5.0 for growing healthy duckweed while controlling the algae. I wasn't sure if you have come across this solution, so I wanted to share.

Thank you,

Sarah Gilbert

PhD Student

Rutgers University

## First International Duckweed Industry Workshop

**Paul Fourounjian (Doctoral student, Rutgers University)**

MamaGrande is a social company founded in 2012 by Federico Seineldin, Sebastian Lagorio and Eduardo Mercovich. The philosophy of a social company is best explained by Eduardo's bicycle metaphor, where the rear wheel- the company's profits provides the momentum, and the front wheel- the benefit to people and the environment, provides the direction.

MamaGrande is growing duckweed on ~155 hectares of municipal wastewater treatment lagoons, the largest duckweed facility in the world. This is a partnership between MamaGrande and Aguas del Norte: a public-private drinking water and wastewater treatment non-profit company.

MamaGrande's final plan to use the duckweed is to ferment it into polylactic acid (PLA). They know how to grow and harvest duckweed easily. They are now working with their fermentation facility to find the reactor conditions to pretreat the biomass to release available starch, grow a bacteria that converts free starch into lactic acid, and then another bacteria that converts lactic acid into polylactic acid. Eager for a new way to approach this challenge and happy to exchange ideas, MamaGrande hosted the First International Duckweed Industry Workshop.

All interested parties from around the world were invited to sunny Argentina in mid-November, and three came. Tamra Fakhorian; Exec. Director of the International Lemna Association, Founder & CEO of GreenSun Products, Mayfield, KY USA and an experienced business woman for the past 30 years. Finland's Markku Taulamo; Founder & former CEO of an IT company Futurice, past investment banker and part-time chemistry student who turned to duckweed due to his love for the natural world. Finally, myself, Paul Fourounjian; Doctoral student of Dr. Joachim Messing, Spirodela miRNA researcher, Rutgers University, USA and one who wishes to join the industry postgraduation. We three flew to Argentina for the workshop where we learned all about MamaGrande, and worked with them to find new solutions and applications to improve profitability.

The three of us met up at the Coloria Hostel in Salta and met with Santiago on the morning of November 15th. Santiago explained the MamaGrande-Aguas del Norte partnership, and took us to three different wastewater treatment lagoons, (WWTL) Moldes, Carril, and Norte. Each one was bigger than the last, and I got to see the largest duckweed lagoon of my life three times that day.



MamaGrande is a company of 5 people:

- Sebastian Cinquini (CEO)
  - Pablo Echevarria (engineering)
  - Santiago Zannier (primary production)
  - Federico Bologna (biology)
  - Rodrigo Funes (lab technician)
- and 4 Founders/Directors;
- Federico Seineldin
  - Paula Cardenau
  - Emiliano Fazio
  - Eduardo Mercovich





From left to right: Markku, Paul and Santiago. Inset in the top right corner, Tamra. All were at PDN (30 has WWT plant)

Planta Depuradora Norte was a large 30ha facility with duckweed, azolla, and even pennywort growing on the 6 secondary lagoons, all bunched tightly thanks to a strong breeze. We rode in MamaGrande's trusty four wheel drive pickup truck through rivers and stream banks at times to reach these remote sites. Due to pond inaccessibility and design, production, harvesting, drying and transporting equipment for the duckweed would be extremely costly; necessitating creative plans on how to use the biomass.

Santiago also took us to Planta Depuradora Sur, a municipal WW treatment plant that treated 70% of the sewage from the city of Salta. We toured a large screening machine, a number of stone and sand filter tanks, a solids settling tank and large anaerobic digesters. There we met Rodrigo, MamaGrande's biologist, who analyzes duckweed and water samples along with other experiments. He gave us a wonderful tour of his lab and mentioned MamaGrande's production and their use of a (now) commercially available grease eating bacillus bacteria in WW treatment. Santiago was kind enough to invite us into his home that night after dinner. The next morning, we flew to Rosario, Argentina.

In Rosario on the 17th we met the rest of the MamaGrande team; Eduardo who founded the company with Federico, Paula and Emiliano, Sebastian -the CEO- who's experienced in supply chain management, and Pablo, who knows multiple types of engineering. Their office is Njambre, which means "swarm" in Spanish. It's a start up incubator managed by Federico Seineldin in the house he grew up in. MamaGrande was founded here, and they share the space with many other startups. We moved quickly from breakfast to data. They had already figured out how to raise 12-60 metric tons/day of wet duckweed, which translated to roughly one to five dry tons/day. Pablo & Sebastian created a wonderful multiple variable regression analysis model in a spreadsheet, where you can adjust any parameter of duckweed growth, starch, protein, and moisture content as well as variable of percent moisture by drying with solar heat, and transportation etc. to see the effect on cost and compare it to corn. This model was crucial in demonstrating that drying and transportation were their two largest costs that needed to be minimized or eliminated.

They then took us to INDEAR, Argentina's largest biotech incubator. There they showed us their two bench top 1L fermenters, as well as 250L, and 1,000L fermenters. These four fermenters are the only benchtop to industry scale research facility in Argentina, which has allowed MamaGrande to rent the facility out to other companies.





From left to right: Paul, Pablo, Tamra and Markku, in a partial panoramic view of the main 200 and 1000 l fermentors, filled to the brim with sensors and actuators.

Additionally, the incubator has a large sequencing facility with well-trained staff. There they showed us their minION sequencer from Oxford Nanopore. This machine is still limited in availability (Beta stage), and NASA is currently testing it in outer space!

Our minds racing from all the data, we went off to plan, sleep and dream. On the 18th we were back at it, with new business models to discuss. After long discussions about each plan's feasibility we finally agreed over dinner on two of them that seemed to be worth investigating on the computer, and then possibly at the bench scale.



DNA sequencers timeline: on the left, an old Roche GS FLX+, on the right into the researcher hands, the Minlon. Invisible on this photo, a couple meters on the back of the photographer, 2 Illumina.



From left: Markku, Paul, Sebastián (MamaGrande's CEO), Tamra, and Pablo discussing ideas in Njambre.

We all saw the workshop as a resounding success with everyone learning a huge amount, and the MamaGrande team turning the bicycle to find additional applications of duckweed to create an interim cash flow. With the workshop complete, we went our separate ways looking forward to the 4<sup>th</sup> International Duckweed Research and Applications Conference in Periyé, and maybe a 2<sup>nd</sup> International Duckweed Industry workshop in the near future as well.

# From the database

## Highlights

### **Comprehensive Definition of Genome Features in *Spirodela polyrhiza* by High-Depth Physical Mapping and Short-Read DNA Sequencing Strategies**

Michael TP , Bryant D , Gutierrez R , Borisjuk N , Chu P , Zhang H , Xia J , Zhou J , Peng H ,

El Baidouri M , Ten Hallers B , Hastie AR , Liang T , Acosta K , Gilbert S , McEntee C , Jackson

SA , Mockler TC , Zhang W , Lam E .

THE PLANT JOURNAL Oct 18. doi: 10.1111/tpj.13400. [Epub ahead of print] (2016)

*Spirodela polyrhiza* is a fast-growing aquatic monocot with highly reduced morphology, genome size and number of protein-coding genes. Considering these biological features of *Spirodela* and its basal position in the monocot lineage, understanding its genome architecture could shed light on plant adaptation and genome evolution. Like many draft genomes, however, the 158 Mbp *Spirodela* genome sequence has not been resolved to chromosomes and important genome characteristics have not been defined. Here we deployed rapid genome-wide physical maps combined with high-coverage short-read sequencing to resolve the 20 chromosomes of *Spirodela* and to empirically delineate its genome features. Our data revealed a dramatic reduction in the number of the rDNA repeat unit in *Spirodela* to less than 100, which is even fewer than that reported for yeast. Consistent with its unique phylogenetic position, small RNA sequencing revealed 29 *Spirodela*-specific miRNA, with only 2 being shared with oil palm and banana. Combining DNA methylation data and small RNA sequencing enabled accurate prediction of 20.5% Long Terminal Repeats (LTRs) that doubled the previous estimate, and revealed a high Solo:Intact LTR ratio of 8.2. Interestingly, we found that *Spirodela* has the lowest global DNA methylation levels (9%) of any tested plant species. Taken together our results reveal a genome that has undergone reduction likely through eliminating non-essential protein coding genes, rDNA and LTRs. In addition to delineating genome features of this unique plant, the methodologies described and large-scale genome resources from this work will enable future evolutionary and functional studies of this basal monocot family.

### **Heterogeneity of cellular circadian clocks in intact plants and its correction under light-dark cycles**

Muranaka, T; Oyama, T

SCIENCE ADVANCES 2, Article Number: e1600500 (2016)

Recent advances in single-cell analysis have revealed the stochasticity and nongenetic heterogeneity inherent to cellular processes. However, our knowledge of the actual cellular behaviors in a living multicellular organism is still limited. By using a single-cell bioluminescence imaging technique on duckweed, *Lemna gibba*, we demonstrate that, under constant conditions, cells in the intact plant work as individual circadian clocks that oscillate with their own frequencies and respond independently to external stimuli. Quantitative analysis uncovered the heterogeneity and instability of cellular clocks and partial synchronization between neighboring cells. Furthermore, we found that cellular clocks in the plant body under light-dark cycles showed a centrifugal phase pattern in which the effect of cell-to-cell heterogeneity in period lengths was almost masked. The inherent heterogeneity in the properties of cellular clocks observed under constant conditions is corrected under light-dark cycles to coordinate the daily rhythms of the plant body. These findings provide a novel perspective of spatiotemporal architectures in the plant circadian system.

## Genome-wide analysis of pentatricopeptide-repeat proteins of an aquatic plant

Wang, WQ; Wu, YR; Messing, J

PLANTA 244: 893-899 (2016)

A large proportion of genes in plant genomes are organized as gene families. Whereas most gene families in the aquatic plant *Spirodela* are reduced in their copy number, the PPR gene family is expanded, which match the RNA editing sites in organelles, providing us with new insights in the evolution of flowering plants. Pentatricopeptide-repeat proteins (PPRs) are nuclear-encoded proteins that are targeted to mitochondria and plastids to stabilize and edit mRNA transcribed from organellar genomes. They have been described for many terrestrial plant species from a diverse spectrum of sequenced genomes. To further increase our understanding of the evolution of this gene family across angiosperms, we analyzed the PPR genes in the aquatic species *Spirodela polyrhiza* in the order of the *Alismatales* (monocotyledonous plants). Because we had generated next generation sequencing data from transcripts and had sequenced the genome of *Spirodela polyrhiza*, we were able to identify its PPR genes and determine the level of their expression. In total, we could identify 556 PPR proteins, of which 238 members belong to the P (P motif) subfamily that is mainly involved in RNA stabilization and 318 ones to the PLS (P, Longer P, shorter P motif) subfamily responsible for RNA editing. Compared to other angiosperms, this is a large increase in the copy number of the PLS-PPRs subfamily and the expansion correlates with the increase of the number of RNA editing sites of organellar transcripts. Expression of PPR was generally stable even during growing and dormant stages, indicating that their function was critical throughout development. However, PPRs, especially those of the PLS subfamily, were expressed at relatively low levels, suggesting a delicate fine-tuning of its trans-acting function in the post-transcriptional regulation of gene expression. Thus, understanding PPR evolution and expression will help decipher the PPR code for their binding sites, which could genetically engineer RNA-binding proteins toward desired sequence.

## Biotechnology

### Composition of the bio-oil from the hydrothermal liquefaction of duckweed and the influence of the extraction solvents

Yan, WH; Duan, PG; Wang, F; Xu, YP

FUEL 185: 229-235 (2016)

The influence of the extraction solvents on the yields of the product fractions and on the composition of the bio-oils obtained from the hydrothermal processing of duckweed at 350 degrees C for 30 min was investigated. Ten solvents were employed including polar solvents (isopropanol, ethyl acetate, dichloromethane, diethyl ether, dichloroethane, benzene, carbon disulfide) and nonpolar solvents (cyclohexane, n-hexane and petroleum ether). The extraction solvents with high relative polarity values tended to produce higher yields of the bio-oils. The highest bio-oil yield of 26 +/- 1 wt.% was obtained using isopropanol, followed by dichloromethane (24 +/- 1 wt.%). Nonpolar solvents including cyclohexane, n-hexane, and petroleum ether produced the yields of the bio-oils ranging from 3 +/- 0.2 to 9 +/- 0.4 wt.%. The bio-oils always had a significantly higher C and H contents and a substantially lower O and S contents than those of the biomass material. The C and H contents of the bio-oil from nonpolar solvents, which averaged 78 +/- 0.8 wt. % and 10 +/- 0.5 wt. %, respectively, were slightly higher than the values from the polar solvents, which averaged 75 wt.% and 9 wt.%, respectively. In contrast, the N and O contents of the bio-oils from nonpolar solvents was lower than that from the polar solvents. The energy recovery (ER) obtained from the polar solvents varied from 42 +/- 2 to 60 +/- 3%, which is much higher than the ER obtained from the nonpolar solvent (24 +/- 1% for cyclohexane) and lowest (10 +/- 0.5% for n-hexane). Significant differences in molecular composition were observed in the bio-oils when varying the solvent, and these differences were attributed to the combined effects of the polarity and the molecular structure of each solvent.

## Physicochemical properties and combustion behavior of duckweed during wet torrefaction

Zhang, SP; Chen, T; Li, W; Dong, Q; Xiong, YQ

BIORESOURCE TECHNOLOGY 218: 1157-1162 (2016)

Wet torrefaction of duckweed was carried out in the temperature range of 130-250 degrees C to evaluate the effects on physicochemical properties and combustion behavior. The physicochemical properties of duckweed samples were investigated by ultimate analysis, proximate analysis, FTIR, XRD and SEM techniques. It was found that wet torrefaction improved the fuel characteristics of duckweed samples resulting from the increase in fixed carbon content, HHVs and the decrease in nitrogen and sulfur content and atomic ratios of O/C and H/C. It can be seen from the results of FTIR, XRD and SEM analyses that the dehydration, decarboxylation, solid-solid conversion, and condensation polymerization reactions were underwent during wet torrefaction. In addition, the results of thermogravimetric analysis (TGA) in air indicated that wet torrefaction resulted in significant changes on combustion behavior and combustion kinetics parameters. Duckweed samples after wet torrefaction behaved more char-like and gave better combustion characteristics than raw sample.

## Ecology

### A comparative study of the nutrient responses of the invasive duckweed *Lemna minuta*, and the native, co-generic species *Lemna minor*

Paolacci, S; Harrison, S; Jansen, MAK

AQUATIC BOTANY 134: 47-53 (2016)

Invasive alien plant species are usually characterized by a growth rate higher than their native competitors, and this higher rate can be achieved through the opportunistic use of plant nutrients. The growth of the invasive alien duckweed *Lemna minuta* and the co-generic native *Lemna minor* were compared under different conditions of nutrient availability. The two species were grown for one week under fully controlled conditions on medium containing increasing concentrations of NO<sub>3</sub>-N, NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub><sup>3-</sup>-P. The effects of different concentrations and ratios of Ca and Mg on growth were also determined. At the end of the experiment the Relative Growth Rate (RGR) of the plants was calculated on the basis of the biomass and number of fronds. The data highlighted that *Lemna minuta* outgrew *L. minor* under conditions of high phosphate supply while, when phosphate concentrations in the medium were low, it grew less than *L. minor*. However, *L. minuta* is not simply an opportunistic species as we observed, relative tolerance to imbalances in Ca-Mg ratio in the growth medium. The different responses observed when the *L. minor* and *L. minuta* were grown at specific concentrations of P, Ca and Mg suggest that these elements are potential determinants of the invasibility of freshwaters and that they should be considered in future field studies.

### Remarkable richness of aquatic macrophytes in 3-years old re-established Lake Fil, Denmark

Baastrup-Spohr, L; Kragh, T; Petersen, K; Moeslund, B; Schou, JC; Sand-Jensen, K

ECOLOGICAL ENGINEERING 95: 375-383 (2016)

In August 2014, we discovered a surprisingly high species richness of aquatic macrophytes (33 species) in Lake Fil only two years after the large, shallow lake had been re-established following land reclamation and 60 years of intense agricultural use. In 2015, plant richness had increased even further (to 40 species). Some rare, oligotrophic species, which formerly were common in the historical lake, have re-established populations (e.g. *Elatine hydropiper* and *Lythrum portula*). However, a larger number of meso- and eutrophic species have colonized the re-established lake, changing the character of aquatic macrophyte flora as compared to the historical lake. The very rare *Baldellia repens*, considered extinct species in Denmark, has become re-established with at least 13

subpopulations in the new Lake Fil. Also two species recently discovered in Denmark (*Callitriche obtusangula* and *Lemna turionifera*) were found. Half of the species may have colonized the re-established lake from now submerged drainage canals and from an upstream lake, while the other half presumably have colonized from more distant lakes. The high richness of aquatic macrophytes in the re-established Lake Fil is matched by only a few natural lakes in Denmark. According to predictions of species richness from environmental conditions and colonized area in 56 natural Danish lakes, the observed richness in Lake Fil is two-fold the expected. We attribute the high plant richness to: shallow waters, large open areas on grazed or physically exposed lake shores and high likelihood of species recruitment from neighboring lakes via dense populations of migratory waterfowl. High plant richness was established despite low transparency due to high release of dissolved organic matter and particles from flooded soils. We conclude that suitable bathymetry, physical disturbance, grazing by cattle and waterfowl in the littoral zone and high dispersal capacity of propagules may ensure rapid establishment of a rich aquatic vegetation in new lakes, even when nutrient-rich farmland is the starting point.

### **Competition between Free-Floating Plants Is Strongly Driven by Previously Experienced Phosphorus Concentrations in the Water Column**

Peeters, ETHM; Neefjes, REM; van Zuidam, BG

PLOS ONE 11: 9 Article Number: e0162780

Nutrients can determine the outcome of the competition between different floating plant species. The response of floating plants to current phosphorus levels may be affected by previously experienced phosphorus concentrations because some species have the ability to store excess phosphorus for later use. This might have an impact on their competition. Here, we investigate the effect of previous and actual phosphorus concentrations on the growth rate of free-floating plant species (*Azolla filiculoides*, *Lemna minor/gibba* and *Ricciocarpus natans*) and the effect of phosphorus history on the competition between *L. minor/gibba* and *A. filiculoides* and between *L. minor/gibba* and *R. natans*. As expected, plant growth was lower when previously kept at low instead of high phosphorus concentrations. Growth of *L. minor/gibba* and *A. filiculoides* with a phosphorus rich history was comparable for low and high actual phosphorus concentrations, however, internal phosphorus concentrations were significantly lower with low actual phosphorus concentration. This indicates that both species perform luxury phosphorus uptake. Furthermore, internal P concentration in *Azolla* and *Lemna* increased within two weeks after a period of P deficit without a strong increase in growth. *A. filiculoides* in a mixture with *L. minor/gibba* grew faster than its monoculture. Morphological differences may explain why *A. filiculoides* outcompeted *L. minor/gibba* and these differences may be induced by phosphorus concentrations in the past. Growth of *L. minor/gibba* was only reduced by the presence of *A. filiculoides* with a high phosphorus history. Growth of *L. minor/gibba* and *R. natans* in mixtures was positively affected only when they had a high phosphorus history themselves and their competitor a low phosphorus history. These observations clearly indicate that phosphorus history of competing plants is important for understanding the outcome of the competition. Therefore, actual and previously experienced phosphorus concentrations should be taken into account in future studies dealing with competition between plants.

### **Arsenic and other heavy metal accumulation in plants and algae growing naturally in contaminated area of West Bengal, India**

Singh, NK; Raghubanshi, AS; Upadhyay, AK; Rai, UN

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 130: 224-233 (2016)

The present study was conducted to quantify the arsenic (As) and other heavy metal concentrations in the plants and algae growing naturally in As contaminated blocks of North-24-Pargana and Nandia district, West Bengal, India to assess their bioaccumulation potential. The plant species included five

macrophytes and five algae were collected from the nine selected sites for estimation of As and other heavy metals accumulated therein by using Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). Results revealed that maximum As concentration (117 mg kg<sup>-1</sup>) was recorded in the agricultural soil at the Barasat followed by Beliaghat (111 mg kg<sup>-1</sup>) sites of North-24-Pargana. Similarly, concentration of selenium (Se, 249 mg kg<sup>-1</sup>), lead (Pb, 79.4 mg kg<sup>-1</sup>), chromium (Cr, 138 mg kg<sup>-1</sup>) was also found maximum in the soil at Barasat and cadmium (Cd, 163 mg kg<sup>-1</sup>) nickel (Ni, 36.5 mg kg<sup>-1</sup>) at Vijaynagar site. Among the macrophytes, *Eichhornia crassipes* found the more dominating species in As contaminated area and accumulate As (597 mg kg<sup>-1</sup>) in the shoot at Kanchrapara site. The *Lemna minor* found to accumulate maximum As (735 mg kg<sup>-1</sup>) in the leaves at Sonadanga and *Pistia stratiotes* accumulated minimum As (24.5 mg kg<sup>-1</sup>) in the fronds from Ranaghat site. In case of diatoms, maximum As (760 mg kg<sup>-1</sup>) was accumulated at Kanchrapara site followed by *Hydrodictyon reticulatum* (403 mg kg<sup>-1</sup>) at the Ranaghat site. High concentration of As and other heavy metal in soil indicates long term effects of irrigation with contaminated ground water, however, high concentration of heavy metals in naturally growing plants and algae revealed their mobilization through leaching and possible food chain contamination. Therefore, efficient heavy metal accumulator macrophytes *Eichhornia crassipes*, *Lemna minor*, *Spirodela polyrhiza* may be exploited in removing metals from contaminated water by developing a plant based treatment system. However, As accumulator algal species may be used as a bioresource for understanding algae mediated As detoxification and bioindication studies.

### **Invasive species *Lemna L. (Lemnaceae)* in the flora of Bulgaria**

Kirjakov, IK; Velichkova, KN

PERIODICUM BIOLOGORUM 118: 131-138 (2016)

During the investigations of flora of Bulgaria new species from genus *Lemna* have been found in Bulgaria. They were conducted during the period 2010 - 2015 for which purpose we explored diverse pools in different parts of Bulgaria (hot and cold swamps, spillages of rivers and streams, hot mineral waters). The species were determined by Flora of North America. In the flora of Bulgaria we found the following new (invasive) species *L. minuta* Kunth., *L. obscura* (Austin) Daubs, *L. perpusilla* Torr. and *L. valdiviana* Philipi. Their morphology, chorology and ecology was recorded and data about the accompanying species are also given. Finding these *Lemna* species in several distant locations in our country is a clear indication that the species is transferred on the territory of Bulgaria a long time ago. The species are most likely invading from neighboring fields in Southeast Europe - Greece, Romania, Turkey and others.

## **Feed & Food**

### **Nutritional value of duckweeds (*Lemnaceae*) as human food**

Appenroth, KJ; Sree, KS; Bohm, V; Hammann, S; Vetter, W; Leiterer, M; Jahreis, G

FOOD CHEMISTRY 217: 266-273 (2017)

Duckweeds have been consumed as human food since long. Species of the duckweed genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia* were analysed for protein, fat, and starch contents as well as their amino acid and fatty acid distribution. Protein content spanned from 20% to 35%, fat from 4% to 7%, and starch from 4% to 10% per dry weight. Interestingly, the amino acid distributions are close to the WHO recommendations, having e.g. 4.8% Lys, 2.7% Met + Cys, and 7.7% Phe + Tyr. The content of polyunsaturated fatty acids was between 48 and 71% and the high content of n3 fatty acids resulted in a favourable n6/n3 ratio of 0.5 or less. The phytosterol content in the fastest growing angiosperm, *W. microscopica*, was 50 mg g<sup>-1</sup>, lipid. However, the content of trace elements can be adjusted by cultivation conditions. Accordingly, *W. hyalina* and *W. microscopica* are recommended for human nutrition.

## Nutrient Value of Leaf vs. Seed

Edelman, M; Colt, M

FRONTIERS IN CHEMISTRY 4, Article Number: 32 (2016)

Major differences stand out between edible leaves and seeds in protein quality, vitamin, and mineral concentrations and omega 6/omega 3 fatty acid ratios. Data for seeds (wheat, rice, corn, soy, lentil, chick pea) are compared with corresponding data for edible green leaves (kale, spinach, broccoli, duckweed). An x/y representation of data for lysine and methionine content highlights the group differences between grains, pulses, leafy vegetables, and animal foods. Leaves come out with flying colors in all these comparisons. The perspective ends with a discussion on "So why do we eat mainly seeds?"

## In vitro digestibility study of some plant protein sources as aquafeed for carps *Labeo rohita* and *Cyprinus carpio* using pH-Stat method

Sharma, JG; Kumar, A; Saini, D; Targay, NL; Khangembam, BK; Chakrabarti, R

INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY 54: 606-611 (2016)

Aquaculture, as a promising food industry, is expected to meet the demand for quality food from the increasing human population. As the diet is critical for feeding farm fish, such a faster growth in the industry is destined to create stress in the fishmeal market to supply diets to the tune. In this context, here, we studied the protein content of 20 plant ingredients, including aquatic weeds, cereals, pulses and oil-cakes using micro-Kjeldahl method and evaluated in vitro digestibility of these ingredients for rohu *Labeo rohita* and common carp *Cyprinus carpio* using pH-Stat method. The protein contents of water fern, duckweed, almond oil-cake and soybean product were 20.81, 39.75, 47.78 and 57.48%, respectively. Species specific digestibility was found for the same plant ingredient. The degree of hydrolysis for water fern, duck weed, almond oil-cake and soybean product were 14.17, 4.80, 17.30 and 3.57%, respectively for rohu and 4.58, 6.03, 12.17 and 3.35%, respectively for common carp. This study showed that incorporation of water fern and almond oil-cake in the diet of rohu, and duckweed and almond oil-cake in the diet of common carp are beneficial considering their protein content and digestibility. These are cost-effective, protein-rich feed ingredients for aquafeed.

## Molecular Biology

### Comparative Analysis of GC Content Variations in Plant Genomes

Singh, R; Ming, R; Yu, QY

TROPICAL PLANT BIOLOGY 9: 136-149 (2016)

The GC content, one of the important compositional features of the genome, varies significantly among different genomes and different regions within a genome. Identifying the driving force that shaped the GC content and deciphering the biological meaning of variations in the GC content will help us to understand genome evolution. We analyzed and compared the GC contents of 20 selected plant species, representing the major evolutionary lineages. Our result revealed the highest GC content and GC heterogeneity in the grass genomes followed by the non-grass monocot and dicot genomes. The detailed analysis of GC content in genic regions showed higher GC content in terminal exons than in internal exons in all selected species except *Volvox carteri*. A strong correlation between the GC contents of exons and their neighboring introns at terminals of genes was observed in all the grasses, *Musa acuminata*, *Spirodela polyrhiza* and *Nelumbo nucifera* genomes. Our result suggested that the widely reported negative gradient of GC3 along the coding sequences from 5' to 3' was likely an artifact caused by GC content calculations on an admixture of genes with variable lengths and exon numbers. Our findings supported the role of the GC biased gene conversion in shaping the nucleotide composition landscapes in monocots. The U shape pattern of the GC content



along the genes may have resulted from variable degrees of interactions among transcription, replication and DNA repair machineries. The transcription-associated recombination might play a major role in GC content evolution.

## Phytoremediation

### **Comprehensive review on phytotechnology: Heavy metals removal by diverse aquatic plants species from wastewater**

Rezania, S; Taib, SM; Din, MFM; Dahalan, FA; Kamyab, H

JOURNAL OF HAZARDOUS MATERIALS 318: 587-599 (2016)

Environmental pollution specifically water pollution is alarming both in the developed and developing countries. Heavy metal contamination of water resources is a critical issue which adversely affects humans, plants and animals. Phytoremediation is a cost-effective remediation technology which able to treat heavy metal polluted sites. This environmental friendly method has been successfully implemented in constructed wetland (CWs) which is able to restore the aquatic biosystem naturally. Nowadays, many aquatic plant species are being investigated to determine their potential and effectiveness for phytoremediation application, especially high growth rate plants i.e. macrophytes. Based on the findings, phytofiltration (rhizofiltration) is the sole method which defined as heavy metals removal from water by aquatic plants. Due to specific morphology and higher growth rate, free-floating plants were more efficient to uptake heavy metals in comparison with submerged and emergent plants. In this review, the potential of wide range of aquatic plant species with main focus on four well known species (hyper accumulators): *Pistia stratiotes*, *Eicchornia spp.*, *Lemna spp.* and *Salvinia spp.* was investigated. Moreover, we discussed about the history, methods and future prospects in phytoremediation of heavy metals by aquatic plants comprehensively.

### **Biogeochemistry of uranium in the soil-plant and water-plant systems in an old uranium mine**

Favas, PJC; Pratas, J; Mitra, S; Sarkar, SK; Venkatachalam, P

SCIENCE OF THE TOTAL ENVIRONMENT 568: 350-368 (2016)

The present study highlights the uranium (U) concentrations in water-soil-plant matrices and the efficiency considering a heterogeneous assemblage of terrestrial and aquatic native plant species to act as the biomonitor and phytoremediator for environmental U-contamination in the Sevilha mine (uraniferous region of Beiras, Central Portugal). A total of 53 plant species belonging to 22 families was collected from 24 study sites along with ambient soil and/or water samples. The concentration of U showed wide range of variations in the ambient medium: 75 to 557 mg kg<sup>-1</sup> for soil and 0.4 to 113 µg L<sup>-1</sup> for water. The maximum potential of U accumulation was recorded in roots of the following terrestrial plants: *Lanais squarrosus* (450 mg kg<sup>-1</sup> DW), *Cortina corymbosa* (181 mg kg<sup>-1</sup> DW) and *Juncus bufonius* (39.9 mg kg<sup>-1</sup> DW), followed by the aquatic macrophytes, namely *Callitriche stagnalis* (55.6 mg kg<sup>-1</sup> DW) *Lemna minor* (53.0 mg kg<sup>-1</sup> DW) and *Riccia fluitans* (50.6 mg kg<sup>-1</sup> DW). Accumulation of U in plant tissues exhibited the following decreasing trend: root > leaves > stem > flowers/fruits and this confirms the unique efficiency of roots in accumulating this radionuclide from host soil/sediment (phytostabilization). Overall, the accumulation pattern in the studied aquatic plants (*L. minor*, *R. fluitans*, *C. stagnalis* and *Lythrum portula*) dominated over most of the terrestrial counterpart. Among terrestrial plants, the higher mean bioconcentration factor (approximate to 1 in roots/rhizomes of *C. corymbosa* and *squarrosus*) and translocation factor (31 in *Anthyala integrifolia*) were encountered in the representing families *Asteraceae* and *Juncaceae*. Hence, these terrestrial plants can be treated as the promising candidates for the development of the phytostabilization or phytoextraction methodologies based on the accumulation, abundance and biomass production.

## Significance of natural treatment systems to enhance reuse of treated effluent: A critical assessment

Kumar, D; Asolekar, SR

ECOLOGICAL ENGINEERING 94: 225-237 (2016)

This paper summarizes the results of the recently completed India-wide survey of 108 wastewater treatment facilities based on natural treatment systems (NTSs) engaged in wastewater treatment and reuse. During assessment of NTSs, a questionnaire survey was administered for collection of vital data on the shortlisted 41 treatment facilities - especially to obtain insights about the technical, economical and social issues influencing the success and failure of the given facility. Further, five case studies based on the most commonly practiced NTSs were selected for in-depth evaluation and the critical issues including effectiveness of technology and the socioeconomic aspects were studied. The treatment performance of these systems was assessed for one year by collecting primary data during January, June and August corresponding to the three seasons viz. winter, summer and monsoon. The effectiveness of the facilities was assessed in terms of percentage removals as well as the mass removal rate for commonly used parameters. As of today, the total load of sewages including sullages serviced by NTSs in India adds up to be around 1838 million liters a day. The most commonly practiced NTS in India has been the waste stabilization ponds (74 facilities) when compared with the phyto-remediation based systems (19 facilities) followed by the polishing ponds (15 facilities). Among the 41 facilities, nearly 75% facilities were compliant and managed to produce treated sewages suitable for irrigation, discharged into wastewater canals or percolated in the riparian zone of the river. All the five systems that were selected for in-depth evaluation were found compliant during assessment. Also, two facilities (Sewage-fed Aquaculture Karnal and Duckweed Pond Ludhiana) were found to be operating on the public private partnership model and generating revenue for operation and maintenance along with ample benefits to the operating agencies. The study also reveals the associated socioeconomic benefits of various practiced NTSs. Finally, strategies for achieving improved performance of NTSs were articulated - especially focusing the potential for recycling and reusing of treated wastewaters.

## Shallow pond systems planted with *Lemna minor* treating azo dyes

Yaseen, DA; Scholz, M

ECOLOGICAL ENGINEERING 94: 295-305 (2016)

A higher demand on textile materials has resulted in an increase of the number of textile factories particularly in the developing world, which consequently negatively effects the environment due to their contaminated effluents. Textile effluents are highly coloured and mixed with different chemicals and pollutants. Shallow pond systems are a promising, cheap and effective technique for the treatment of contaminated wastewater. The aim of this study is to assess the performance of pond systems vegetated by *Lemna minor* L. (duckweed) for textile dye removal under controlled laboratory conditions. The key objectives of this study are to assess the influence of design variables on water quality parameters, the dye and chemical oxygen demand (COD) removal of dyes, and the effect of dye accumulation as a function of the relative growth rate of *L. minor*. Findings indicate that the simulated shallow pond system (as a polishing step) is able to remove only Basic Red 46 (BR46) in low concentrations, and ponds containing *L. minor* significantly ( $p < 0.05$ ) outperformed algae-dominated ponds and control ponds. The simple chemical structure, absence of sulpho-group and small molecular weight associated with neutral pH values enhanced the capacity of the uptake of BR46 molecules. Furthermore, the total dissolved solid concentrations were within the threshold set for discharge to the aquatic environment.

## Bioremediation of PCB-contaminated sediments and evaluation of their pre- and post-treatment ecotoxicity

Dudasova, H; Laszlova, K; Lukacova, L; Balascakova, M; Murinova, S; Dercova, K

CHEMICAL PAPERS 70: 1049-1058 (2016)

The paper deals with the application of two perspective and promising bioremediation approaches, bioaugmentation and biostimulation, applied to sediment contaminated with polychlorinated biphenyls (PCBs) with the aim to enhance biodegradation of PCBs. Sediments were sampled from Strazsky canal, an industrial waste canal that flows from a former PCB-factory through the Laborec river into the Zemplinska sirava water reservoir, all located in the eastern part of Slovakia.

Bioaugmentation of sediments was performed in microcosms using two bacterial isolates with PCB-degradation ability obtained from the contaminated sediment: *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*. Biostimulation was performed using an addition of cut plants containing terpenes, known as PCB-inducers (ivy leaves and pine needles). Ecotoxicity of the contaminated sediments was evaluated pre- and post-treatment using biotests of the standard aquatic plant *Lemna minor*, standard contact test using *Lactuca sativa* var. *capitata*, and the bioluminescent bacteria *Vibrio fischeri*. Biostimulation treatment using ivy leaves revealed higher degradation of detected PCB congeners than that achieved by the addition of pine needles, but moderately higher post-treatment toxicity of the sediment to the bioindicator *Lemna minor*.

### **Potential demand for recoverable resources from Indonesian wastewater and solid waste**

Kerstens, SM; Priyanka, A; van Dijk, KC; De Ruijter, FJ; Leusbrock, I; Zeeman, G

RESOURCES CONSERVATION AND RECYCLING 110: 16-29 (2016)

Projected population growth and urbanization will become a challenge for finite natural resources, their distribution and local availability. At the same time, 2.5 billion people do not have access to sanitation facilities. Indonesia is one of these rapidly growing countries with a poorly developed municipal wastewater and solid waste sector. Without an integrating concept to recover and reuse resources, "waste flows" are discarded and their potential value is ignored. Therefore, the Indonesian backlog may be an opportunity, since it allows for direct introduction of a circular resource approach. To foster a sustainable municipal wastewater and solid waste management, the 20 years' demand forecast of recoverable resources (phosphorus, compost, duckweed, plastic and paper) was analyzed. Phosphorus, compost and duckweed analysis was based on nutritional demand and not on market demand. Demand for recoverable plastic and paper related to the potential substitution of conventionally manufactured products. Phosphorus and compost demand analysis was based on (1) fertilizer requirements of 68 crops (staple food, horticulture and plantation), and (2) anticipated increase in production area of these crops. Duckweed demand as a protein-rich fish feed was analyzed based on the forecasted demand from aquaculture (tilapia and carp). The potentially recoverable (waste) plastic and paper to substitute conventional manufactured products were based on extrapolation of past trends in plastic and paper production in Indonesia. The potential contribution of recoverable, products to the forecasted demand for 2035 was assessed for phosphorus (15%), compost (35%), duckweed (7%), plastic (66%) and paper (18%). A geographical discrepancy between potential recovery and demand location for phosphorus and compost was found. Therefore, the locations of potential markets should be considered in the planning and selection of wastewater and solid waste facilities. The presented methodology to assess the potential demand for recoverable resources from wastewater and solid waste may be applied in other countries as well.

## **Phytotoxicology**

### **Aquatic toxicity structure-activity relationships for the zwitterionic surfactant alkyl dimethyl amine oxide to several aquatic species and a resulting species sensitivity distribution**

Belanger, SE; Brill, JL; Rawlings, JM; McDonough, KM; Zoller, AC; Wehmeyer, KR

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 134: 95-105 (2016)

Amine oxide (AO) is a cationically charged surfactant at environmental pH and has previously been

assessed in the OECD (Organization for Economic Cooperation and Development) High Production Volume (HPV) chemicals program. Typical of cationic chemicals, AO is highly aquatically toxic. In this study we vastly improve the knowledge of AO toxicity by developing acute Quantitative Structure Activity Relationships (QSARs) for an alga (*Desmodesmus subspicatus*), an invertebrate (*Daphnia magna*) and a fish (*Danio rerio*) using the appropriate array of OECD Test Guidelines. A chronic toxicity QSAR was also determined for the most sensitive taxon, *Desmodesmus*. Pure AO spanning the chain lengths of C8 to C16 were tested individually with trace analytical confirmation of exposures in all tests. The QSARs were all of high quality (R<sup>2</sup> 0.92-0.98) with slopes ranging from -0.338 to -0.484. QSARs were then used to normalize toxicity outcomes for a larger, previously published data set used in HPV, European REACH (Registration, Evaluation, and Authorization of Chemicals), and peer reviewed publications. Two additional species, *Lemna gibba* (macrophyte) and *Ankistrodesmus falcatus* (alga) were studied in exposures to dodecyl (C12) AO to provide sufficient taxonomic diversity to conduct a Species Sensitivity Distribution (SSD) analysis. The SSD 5th percentile hazardous concentration (HC5) to C12 AO was found to be 0.052 mg/L which is similar to an existing AO 28-d, 3-community periphyton community bioassay normalized to C12 AO (No-observed-effect-concentration or NOEC = 0.152 mg/L). The statistical properties of the SSD was probed suggesting that new studies of additional taxa would be required that were at least 10-fold more sensitive than the most sensitive taxon to move the HC5 lower by a factor of 3. The overall AO hazard assessment suggests a large margin of safety relative to published environmental exposure data.

### **Environmental behavior of the chiral insecticide fipronil: Enantioselective toxicity, distribution and transformation in aquatic ecosystem**

Qu, H; Ma, RX; Liu, DH; Gao, J; Wang, F; Zhou, ZQ; Wang, P

WATER RESEARCH 105: 138-146 (2016)

The enantioselective environmental behaviors of the chiral insecticide fipronil and its metabolites in lab scale aquatic ecosystems were studied and the toxicity of fipronil enantiomers and the metabolites to non-target organisms *Lemna minor* (*L. minor*) and *Anodonta woodiana* (*A. woodiana*) was also investigated in this work. Water-sediment, water-*L. minor*, water-*A. woodiana*, and water-sediment-*L. minor*-*A. woodiana* ecosystems were set up and exposed to fipronil through a 90-day period. The results showed fipronil could be degraded significantly faster (half-life of 4.6 days) in the complex water-sediment-*L. minor*-*A. woodiana* ecosystem. *A. woodiana* played a crucial role in the dissipation of fipronil, and the microorganisms in the sediment also made great contribution to the degradation of fipronil in aquatic ecosystems. All the three metabolites fipronil desulfinyl, fipronil sulfide and fipronil sulfone were detected in the ecosystems and were more persistent than fipronil. Enantioselective degradation of fipronil was observed with S-fipronil being preferentially degraded in sediment and *L. minor*, while R-fipronil was metabolized preferentially in *A. woodiana*. EC<sub>50</sub> for *L. minor* was obtained using 7-day exposure, and for *A. woodiana* was obtained using 72-h exposure. S-fipronil was more toxic to *A. woodiana*, while R-fipronil showed higher toxicity to *L. minor*. Moreover, the three metabolites were found more toxic than fipronil indicating significant environment risks due to their persistence. The present study might have important implications for the risk assessment of fipronil and its metabolites in real aquatic environment.

### **Getting More Ecologically Relevant Information from Laboratory Tests: Recovery of *Lemna minor* After Exposure to Herbicides and Their Mixtures**

Knezevic, V; Tunic, T; Gajic, P; Marjan, P; Savic, D; Tenji, D; Teodorovic, I

ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 71: 572-588 (2016)

Recovery after exposure to herbicides-atrazine, isoproturon, and trifluralin-their binary and ternary mixtures, was studied under laboratory conditions using a slightly adapted standard protocol for

*Lemna minor*. The objectives of the present study were (1) to compare empirical to predicted toxicity of selected herbicide mixtures; (2) to assess *L. minor* recovery potential after exposure to selected individual herbicides and their mixtures; and (3) to suggest an appropriate recovery potential assessment approach and endpoint in a modified laboratory growth inhibition test. The deviation of empirical from predicted toxicity was highest in binary mixtures of dissimilarly acting herbicides. The concentration addition model slightly underestimated mixture effects, indicating potential synergistic interactions between photosynthetic inhibitors (atrazine and isoproturon) and a cell mitosis inhibitor (trifluralin). Recovery after exposure to the binary mixture of atrazine and isoproturon was fast and concentration-independent: no significant differences between relative growth rates (RGRs) in any of the mixtures (IC10(Mix), 25(Mix), and 50(Mix)) versus control level were recorded in the last interval of the recovery phase. The recovery of the plants exposed to binary and ternary mixtures of dissimilarly acting herbicides was strictly concentration-dependent. Only plants exposed to IC10(Mix), regardless of the herbicides, recovered RGRs close to control level in the last interval of the recovery phase. The inhibition of the RGRs in the last interval of the recovery phase compared with the control level is a proposed endpoint that could inform on reversibility of the effects and indicate possible mixture effects on plant population recovery potential.

### **Oxidative stress in duckweed (*Lemna minor* L.) induced by glyphosate: Is the mitochondrial electron transport chain a target of this herbicide?**

Gomes, MP; Juneau, P

ENVIRONMENTAL POLLUTION 218: 402-409 (2016)

We investigated the physiological responses of *Lemna minor* plants exposed to glyphosate. The deleterious effects of this herbicide on photosynthesis, respiration, and pigment concentrations were related to glyphosate-induced oxidative stress through hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation. By using photosynthetic and respiratory electron transport chain (ETC) inhibitors we located the primary site of reactive oxygen species (ROS) production in plants exposed to 500 mg glyphosate l(-1). Inhibition of mitochondrial ETC Complex I by rotenone reduced H<sub>2</sub>O<sub>2</sub> concentrations in glyphosate-treated plants. Complex HI activity was very sensitive to glyphosate which appears to act much like antimycin A (an inhibitor of mitochondrial ETC Complex III) by shunting electrons from semiquinone to oxygen, with resulting ROS formation. Confocal evaluations for ROS localization showed that ROS are initially produced outside of the chloroplasts upon initial glyphosate exposure. Our results indicate that in addition to interfering with the shikimate pathway, glyphosate can induce oxidative stress in plants through H<sub>2</sub>O<sub>2</sub> formation by targeting the mitochondrial ETC, which would explain its observed effects on non-target organisms.

### **Ecotoxicological assessment of biosolids by microcosms**

Groth, VA; Carvalho-Pereira, T; da Silva, EM; Niemeyer, JC

CHEMOSPHERE 161: 342-348 (2016)

Biosolids have been applied as soil amendments to improve and maintain the soil fertility and faster plant growth. In spite of its beneficial use, the potential risks of land disposal should be analyzed, considering potential ecological receptors in soil and water. This work describes the use of an early warning laboratory microcosm system to evaluate the integrated ecotoxicological potential of two biosolids: BIO-1 and BIO-2 (18 and 28 months after landfarming, respectively), from an effluent treatment station in a petrochemical and industrial district. The endpoints related to habitat function were: a) germination, growth and biomass of *Phaseolus vulgaris*; b) survival, biomass and number of cocoons of *Eisenia andrei* (*Oligochaeta*) and; c) reproduction of *Folsomia candida* (*Collembola*). The retention function was evaluated by testing the leachates using the tropical cladoceran *Latonopsis australis* (*Cladocera*) in a 48-h acute toxicity test, and growth of the aquatic plant *Lemna minor* in a 7-d chronic test. Tropical artificial soil (TAS) and a natural soil (NS) from the region were used as control soils. Results showed no chronic toxicity of BIO-1 and BIO-2 to the soil organisms tested, but acute toxicity of BIO-1 in the leachate for 50% of *L. australis*, and chronic toxicity of both biosolid

leachates to *L. minor* (inhibition of growth rate), indicating potential risks to aquatic ecosystems. The results confirmed the ability of this microcosm system as a rapid tool to assess biosolid toxicity over time and its potential for hazardous waste characterization in environmental risk assessment, in a screening phase.

### **A full evaluation for the enantiomeric impacts of lactofen and its metabolites on aquatic macrophyte *Lemna minor***

Wang, F; Liu, DH; Qu, H; Chen, L; Zhou, ZQ; Wang, P

WATER RESEARCH 101: 55-63 (2016)

Pesticide pollution of surface water represents a considerable danger for the aquatic plants which play very crucial roles in aquatic system such as oxygen production, nutrient cycling, water quality controlling and sediment stabilization. In this work, the toxic effects of the chiral herbicide lactofen and its three metabolites (desethyl lactofen, acifluorfene and amino acifluorfene) to the aquatic plant *Lemna minor* (*L. minor*) on enantiomeric level were evaluated. The influences on growth rate, fresh weight, content of photosynthetic pigment, protein and malondialdehyde (MDA) and the activities of antioxidant defense enzymes (catalase (CAT) and superoxide dismutase (SOD)) were measured after 7 days of exposure. *L. minor* growth was inhibited in the order of (S)-desethyl lactofen > racemic-desethyl lactofen > (R)-desethyl lactofen > racemic-lactofen > (S)-lactofen > (R)-lactofen > acifluorfene > amino acifluorfene, and the IC<sub>50</sub> (7d) values showed desethyl lactofen was the most powerful compound which was about twice as toxic as lactofen. The contents of chlorophylls (Chl) and carotenoids (Car) were significantly reduced by the chemicals, while, the levels of protein, MDA and the activity of CAT and SOD enzymes increased in most cases. The obtained results revealed that lactofen and its metabolites had an undesirable effect on *L. minor*, in terms of physiological and biochemical aspects. Besides, enantioselective toxicity of lactofen and desethyl lactofen to *L. minor* was observed. The S-enantiomer of desethyl lactofen was more toxic than the corresponding R-enantiomer. Furthermore, racemic lactofen was more toxic than the individual enantiomers. The side effects of pesticide metabolites and the enantioselectivity should be considered in developing optically pure products and risk assessment.

### **Acute toxicity and environmental risk of oxytetracycline antibiotic for tilapia (*Oreochromis niloticus*), *Daphnia magna*, and *Lemna minor***

Machado, AA; Americo-Pinheiro, JHP; Carraschi, SP; Cruz, C; Machado-Neto, JG

ARQUIVO BRASILEIRO DE MEDICINA VETERINARIA E ZOOTECNIA 68: 1244-1250 (2016)

The aim of this study was to classify the antibiotic Terramycin (R) according to acute toxicity and the environmental risk that it poses for *Oreochromis niloticus*, *Daphnia magna*, and *Lemna minor* based on its active ingredient oxytetracycline (OTC). In addition, the occurrence of acute poisoning signs in fish and antibiotic dilution effect in the water quality variables were observed. For this purpose, fingerlings, neonates, and while were exposed to the concentrations of OTC. According to OTC acute toxicity test results, the Terramycin (R) was classified by acute toxicity and environmental poisoning risk classes. To *O. niloticus*, the calculated LC<sub>(l) 50</sub>; 48h was 6.92 mg L<sup>-1</sup>, for *D. magna* the EC<sub>(l) 50</sub>; 48h was 0.17 mg L<sup>-1</sup>, while for *L. minor*, IC<sub>(l) 50</sub>; 7d was 0.68 mg L<sup>-1</sup>. Terramycin (R) was classified as very toxic to *O. niloticus*, and highly toxic to *D. magna* and *L. minor* and cause risk of environmental poisoning for the three organisms tested. Concentrations of 7.5 and 8.0 mg L<sup>-1</sup> OTC reduce the concentration of dissolved oxygen in the water. According to this study, Terramycin (R) should not be used in aquaculture, as it is highly toxic and causes risk of environmental toxicity test organisms.

### **Effects of selenite on chlorophyll fluorescence, starch content and fatty acid in the duckweed *Landoltia punctata***

Zhong, Y; Li, Y; Cheng, JJ

JOURNAL OF PLANT RESEARCH 129: 997-1004 (2016)

Developing a Se-enriched feed for animal has become a considerable effort. In this study, *Landoltia punctata* 7449 was grown over a 12 day period under concentrations of selenite ( $\text{Na}_2\text{SeO}_3$ ) from 0 to 80  $\mu\text{mol L}^{-1}$ . The growth rate, the chlorophyll fluorescence, the starch content and fatty acid were measured. Se at low concentrations of accuracy sign20  $\mu\text{mol L}^{-1}$  had positive effects also on growth rate, fatty acid content and yield of the *L. punctata*. The appropriate Se treatment enhanced the activity of the photosynthetic system by increasing Fv, Fm, Fv/Fm and Fv/Fo and decreasing Fo. However, negative impact to the *L. punctata* was observed when the duckweed was exposed to high Se concentrations (aeyen40  $\mu\text{mol L}^{-1}$ ). Significant increases in starch content in the duckweed were observed after Se application. The present study suggests that the changes in growth rate, the photosynthetic system, the starch content and the fatty acid were closely associated with the application of Se. An increased Se concentration (0-20  $\mu\text{mol L}^{-1}$ ) in duckweed could positively induce photosynthesis, thereby increasing the yield of *L. punctata* and could be a resource for high nutritive quality Se-enrich feed.

### **Effects of selenium on biological and physiological properties of the duckweed *Landoltia punctata***

Zhong, Y; Cheng, JJ

PLANT BIOLOGY 18: 797-804 (2016)

Duckweed can be used for bioremediation of selenium (Se) polluted water because of its capability of absorbing minerals from growing media. However, the presence of Se in the media may affect the growth of the duckweed. *Landoltia punctata* 7449 has been studied for its changes in chemical and biological properties with the presence of Se in the media. The duckweed was cultivated over a 12-day period at different initial concentrations of selenite ( $\text{Na}_2\text{SeO}_3$ ) from 0 to 80  $\text{mol}(-1)$ . The growth rate, the organic and total Se contents, the activity of antioxidant enzymes, the photosynthetic pigment contents, the chlorophyll a fluorescence OJIP transient, and the ultrastructure of the duckweed were monitored during the experiment. The results have shown that Se at low concentrations of 20  $\text{mol}(-1)$  promoted the growth of the *L. punctata* and inhibited lipid peroxidation. Substantial increases in duckweed growth rate and organic Se content in the duckweed were observed at low Se concentrations. The anti-oxidative effect occurred likely with the increases in guaiacol peroxidase, catalase and superoxide dismutase activities as well as the amount of photosynthetic pigments. However, negative impact to the duckweed was observed when the *L. punctata* was exposed to high Se concentrations (40  $\text{mol}(-1)$ ), in which the duckweed growth was inhibited by the selenium. The results indicate that *L. punctata* 7449 can be used for bioremediation of selenium (Se) polluted water when the Se concentration is 20  $\text{mol}(-1)$ .

### **Investigation of Pb species in soils, celery and duckweed by synchrotron radiation X-ray absorption near-edge structure spectrometry**

Luo, LQ; Shen, YT; Liu, J; Zeng, Y

SPECTROCHIMICA ACTA PART B-ATOMIC SPECTROSCOPY 122: 40-45 (2016)

The Pb species play a key role in its translocation in biogeochemical cycles. Soils, sediments and plants were collected from farmlands around Pb mines, and the Pb species in them was identified by X-ray absorption near-edge structure spectrometry. In soils,  $\text{Pb-5}(\text{PO}_4)(3)\text{Cl}$  and  $\text{Pb-3}(\text{PO}_4)(2)$  were detected, and in sediments, Pb-fulvic acids (FAs) complex was identified. A Pb complex with FA fragments was also detected in celery samples. We found that (1) different Pb species were present in soils and sediments; (2) the Pb species in celery, which was grown in sediments, was different from the species present in duckweed, which grew in water; and (3) a Pb-FA-like compound was present in celery roots. The newly identified Pb species, the Pb-FA-like compound, may play a key role in Pb tolerance and translocation within plants.

## MACROPHYTE BIOASSAY APPLICATIONS FOR MONITORING PESTICIDES IN THE AQUATIC ENVIRONMENT

Della Vechia, JF; Cruz, C; Silva, AF; Cerveira, WR; Garlich, N

PLANTA DANINHA 34: 597-603 (2016)

The objective of this study was to evaluate the feasibility of the use of macrophytes *Lemna minor* and *Azolla caroliniana* as biomarkers of exposure (acute toxicity) for atrazine, bentazon + imazamox and clomazone, insecticide alpha cypermethrin + teflubenzuron and fungicides pyraclostrobin and mixture pyraclostrobin + epoxiconazole, as well as the risk for environmental intoxication. For this purpose, four plants of *L. minor* and five of *A. caroliniana* were selected in a 50 mL Hoagland medium. For both plants, the following concentrations were used: 0.10; 1.07; 3.44; 11.16; 36.40 and 118.0 mg L<sup>-1</sup> of each test product and a control with three replicates. The results of this study demonstrated higher sensitivity of *L. minor* to the tested pesticides, when compared to *A. caroliniana*. *L. minor* can be used in the monitoring of herbicides bentazon, atrazine and clomazone and pyraclostrobin-based fungicide, and *A. caroliniana* can be used for pyraclostrobinbased fungicides, due to the sensitivity of these organisms when exposed.

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

Zou, XY; Li, PH; Huang, Q; Zhang, HW

AQUATIC TOXICOLOGY 176: 97-105 (2016)

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





## Links for Further Reading

<http://www.rduckweed.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 Fakhorian

<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.

<http://Lemnopedia.org> Online developing compendium of duckweed research & applications, founded by the ISCDRA.

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