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

# DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 6 (2), issue 21, pages 48-86 (2018)

# Cover page

The cover of this issue of the Duckweed Forum features four Wolffia species that are endemic to particular regions of the world, while predominantly located in warmer climates. ***Wolffia neglecta*** (clone 8917 from Pakistan), have been found in Pakistan, India and Sri Lanka. ***Wolffia borealis*** (clone 9123 from California, USA) is found distributed in temperate regions of North America with milder winters and not too severe summers. Interestingly, recent genotyping results (Bog et al. 2013) indicate that these two species are closely related to each other in spite of their geographical separation. It is interesting to point out that both of these species are also phylogenetically close to the cosmopolitan *W. globosa* in comparison to the other Wolffia species. Thus, it may be possible that *W. neglecta* and *W. borealis* have arose independently from a common ancestor that they share with *W. globosa*. ***Wolffia columbiana*** (clone 9356 from Zulia, Venezuela) is found in temperate regions of both North and South America while ***Wolffia elongata*** (clone 9197 from Cordoba, Colombia) represent a species that has been found only in tropical regions around the northwestern part of South America that has warm and relatively dry climate. The avid production of new daughters can be readily seen in 3 out of the 4 pictures shown with the newly separated daughter already producing a grand-daughter frond in the picture for *W. elongata*. Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

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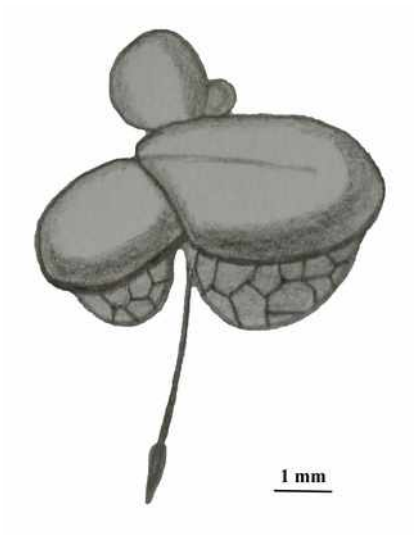
- **External Advisor: Tamra Fakhoorian**, International Lemna Association, Mayfield, KY, USA; tamraf9@gmail.com

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

## Science meets art: *Lemna gibba* L.




*Lemna gibba* L. is the most frequently used duckweed species in phytotoxicity investigations, from the whole plant family Lemnaceae and is a model plant when flower induction should be investigated (cf. L. Fu et al. Scientific Reports 7: 3047 DOI:10.1038/s41598-017-03240-8 (2017)). The names-giving "gibbosity" creates a special aesthetic effect, which makes it easy to recognize this species. Unfortunately, this morphological marker is often not very well developed. As a consequence, *L. gibba* and *L. minor* are frequently interchanged. R. Kandeler focused especially on the delineation of *L. gibba* and *L. minor* (Aquatic Botany 1: 365 (1975)) and wrote: "Hegelmaier was familiar with the fact that fronds of *L. gibba* can vary in a very pronounced manner and that there exist flat forms of *L. gibba* of great similarity with *L. minor*". Nowadays, use of molecular taxonomy instead of morphological markers is recommended when these two species have to be distinguished. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala.

# Letter from the Editor

Dear Duckweed Community,

On behalf of the International Steering Committee on Duckweed Research and Applications, it is my pleasure to bring you our warm greetings in this Spring of 2018 and the new Duckweed Forum issue.

We start this issue of our community newsletter with microscopy pictures for four species of *Wolffia* on the Cover Page. Their beauty in smallness and avid reproductive capability is showcased. After the artistic rendering of *Lemna gibba* in the Science-meets-Art corner by Sowjanya Sree, we bring you the first announcement from the organizers of the 5th ICDRA, followed with online access information for the Abstract Book of the 4th ICDRA by the Frontiers publisher. In the Methods section, a fine contribution on a statistics software ToxRat is provided by Monika Ratte of Germany, with an Introduction by Klaus Appenroth. This should be of especial interest among those of us interested in ecotoxicology. Next is a Student Spotlight on Mr. Barak Cohen from Israel, who carries out duckweed-related research at the Weizmann Institute in Rehovot. Lastly, our current issue ends with the Database section with new titles and abstracts for publications relevant to duckweed research in the past months. As in the past issues, this list was compiled by Klaus Appenroth. I am sure all of you will find this section useful to keep track of the productivity and advances in duckweed research, as I have.

My very best wishes to everyone,

Eric Lam, Chair of the ISCDRA

# 5th ICDRA:1st Announcement

1st Announcement

5th ICDRA International Conference

## Duckweed Research & Applications

at

Weizmann Institute of Science, Rehovot, Israel

**September 9- 12, 2019**

**Save the date**

The Weizmann Institute of Science is set in a lushly landscaped campus in the university town of Rehovot, 25 min. from Tel Aviv and 50 min. from Jerusalem. It is host to 240 experimental and theoretical research groups across five faculties—Biology, Biochemistry, Chemistry, Physics and Mathematics/Computer Science, and to 1400 advanced degree students and postdoctoral fellows.

Accommodations for visitors and conventions exist on campus and at a leading international hotel located 5 min. walk from campus.

**Information & registration for 2nd announcement:**
inbal.azoulay@weizmann.ac.il

# 4th ICDRA: Abstract book

We are pleased to announce that the abstract book of the 4th International Conference on Duckweed Research and Applications at Central University of Kerala, India from 23-26 October, 2017 has been published by Frontiers and it can be accessed on the following web page:

https://www.frontiersin.org/books/4th_International_Conference_on_Duckweed_Research_and_Applications/1524

# Useful methods

Phytotoxicity is one of the fields of duckweed research currently represented by far more scientific publications than any others. One can screen this easily by reading through the section “From the database” in the several issues of our newsletter “Duckweed Forum”. Meanwhile, it is not any more satisfying just to report inhibitory effects of heavy metals, herbicides or other xenobiotics on growth of duckweeds evaluated by frond number, fresh weight or dry weight. As a minimal requirement, these inhibitory effects should be quantified. From effect curves (e.g. number of fronds over the time of treatment depending on the concentrations) and calculating the different exponential growth rates, dose-response-curves or dose-inhibition-curves can be created, commonly as log dose versus inhibitory effects. However, this must not be the end of the evaluation but just the beginning. With suitable statistical methods, effective concentrations (EC) can be calculated together with suitable form of errors. Depending on the requirement of the field, EC values should be calculated at different effect levels like 50 or 10 percent of inhibition. Only such quantitative data offer the opportunity to compare the toxic effects of different herbicides or other substances and also of physical effects like UV or radioactive irradiations. Also, when the sensitivity of different species or strains of duckweeds should be compared, such quantitative evaluation is indispensable. One programme, called ToxRat®, which can be used for such evaluations (and other applications) is explained in the following by Monika Ratte. Additionally to the references given there, we recommend the following papers that show applications in the field of duckweeds:

**Growth rate based dose-response relationships and EC-values of ten heavy metals using the duckweed growth inhibition test (ISO 20079) with *Lemna minor* L. clone St.** Naumann, B; Eberius, M; Appenroth, KJ. (2007) JOURNAL OF PLANT PHYSIOLOGY 164: 1656-1664; DOI: 10.1016/j.jplph.2006.10.011

**Effects of Nickel on the chloroplasts of the duckweeds *Spirodela polyrhiza* and *Lemna minor* and their possible use in biomonitoring and phytoremediation.** Appenroth, KJ; Krech, K; Keresztes, A; Fischer, W; Koloczek, H (2010) CHEMOSPHERE 78: 216-223; DOI: 10.1016/j.chemosphere.2009.11.007

**Klaus-J. Appenroth** (Klaus.Appenroth@uni-jena.de)

Friedrich Schiller University of Jena, Germany

## ToxRat: Custom-designed Software for Statistical Evaluation of Duckweed Growth Inhibition Test

Monika Ratte (monika.ratte@toxrat.com)

ToxRat Solutions GmbH & Co. KG, Naheweg 15, D-52477 Alsdorf, Germany; Phone: +49 2404676767

Duckweed species are used in ecotoxicology to determine the toxic effect of chemicals or waste water on aquatic plants. During the course of usually seven days, frond numbers, frond areas or biomass are determined in different test concentrations and in a control. Growth rates are calculated and the growth rates obtained in treatments are compared to those measured in the control.

## Biology meets statistics

Subsequently, the data needs to be statistically evaluated. Specific effect concentrations causing x% inhibition are calculated by regression methods and/or so called No Observed Effect Concentrations (NOEC) are determined by statistical testing.

Numerous statistical packages are available to perform these evaluations. However, these usually are designed for a comprehensive application range, rather than exclusively for ecotoxicology, not to say especially for the Duckweed growth inhibition test.

## From biologists for biologists

This was the situation in 2002, when Hans Toni and Monika Ratte, two biologists from the Technical University of Aachen, felt the necessity of an interface between ecotoxicology and statistics. "Our vision was to enable both routine evaluation and correct statistical evaluation of biotests even for not-statisticians" says Monika Ratte, who takes care for the customer support. As a result, the software ToxRat was developed: a user-friendly software for the practitioner who wants to perform statistical analysis of biotests according to international guidelines (Figure 1).


Figure 1: Start screen of the software

The programming work has been and is still done by Hans Toni Ratte, associate Professor at the Institute for Environmental Research of the Technical University of Aachen until 2011. Here, he was involved in statistical education of students and attended a lot of boards and committees as expert for ecotoxicology and statistics.

### What makes ToxRat different to other statistical program packages?

### 1. ToxRat "knows" the data structure and experimental design of individual biotests.

When starting the program, the user simply selects a *biotest* from a list of available templates. E.g., for Duckweed data, the user might select either the Lemna Growth Inhibition Test according to OECD 221 (2006) or the Duckweed Growth Inhibition Test according to DIN ISO 20079 (2005) (Figure 2).

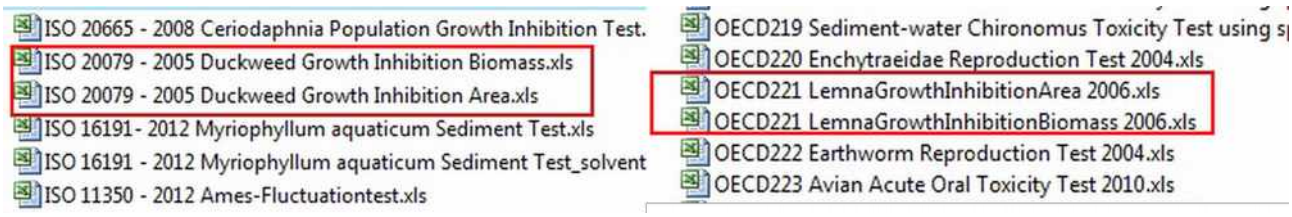



Figure 2: List of data templates included in the terms of delivery (extract)

Thereby a tailor made template for data input will be opened, predesigned to the data recorded in a Duckweed biotest (Figure 3). The template actually is an MS-Excel file, containing different sheets: "General notes" for basic information about the experiment, test item, unit of test item concentration etc. and several sheets for raw data, such as frond number, frond area and biomass. The treatments

are ordered in columns, starting with the control, the replicates are ordered in lines. All general terms such as variable name, number of replicates and even the measurement intervals have been pre-set in accordance with the corresponding guideline (but of course might be edited and changed, if desired). So, the user can easily enter the measurement results directly into the data template or transfer the data via copy-paste.

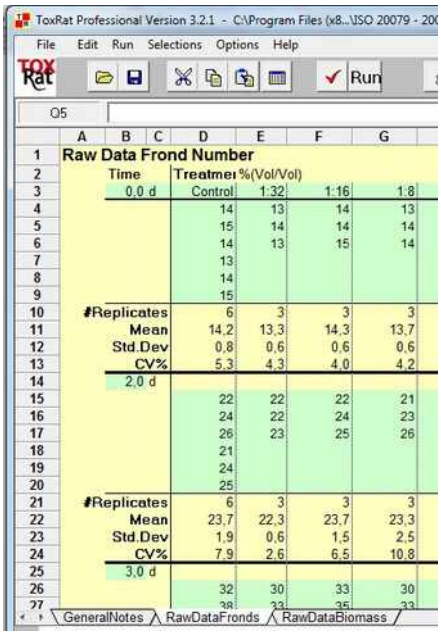



Figure 3: Screenshot from a data template for data obtained in a Duckweed biotest


Figure 4: ToxRat´s "brain": The RUN-Button

| Criterion | Required | User | Achieved | Valid |
|---|---|---|---|---|
| Validity time perioc | 7,0 d | 7,0 d | 7,0 d | yes |
| BF 0 - 7 d | 7 | 7 | 7,6 | yes |
| Doubling time | 2,5 d | 2,5 d | 2,4 d | yes |
| Mean growth rate | 0,275/d | 0,275/d | 0,289/d | yes |

Figure 5: Automatic validity check

2. ToxRat also "knows" biotest guidelines, e.g., which toxic metrics are required by the guideline and which validity criteria are prescribed. As a result, the user doesn´t need to select any statistical method or to perform any mathematical calculation, but can make use of tailor made default settings for the Duckweed growth inhibition test: Pressing the RUN Button (Figure 4), starts a complete evaluation sequence, including validity check (Figure 5), calculation of yields and growth rates from the raw data, percentages of inhibition, regression for ECx calculation and multiple testing just as prescribed in the corresponding guideline. Thereby, up to six user defined EC levels can be selected (Figure 6). For these, also the 95% confidence limits are calculated (Figure 7). Finally, ToxRat generates a comprehensive report with graphics and tables as an rtf or pdf file.

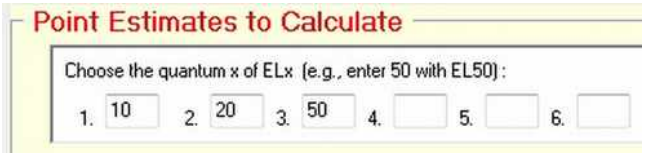



Figure 6: Up to 6 user defined ECx levels

| Toxicity Metric | EC10 | EC20 | EC50 |
|---|---|---|---|
| Value [mg/l] | 8,415 | 18,491 | 83,381 |
| lower 95%-cl | 5,115 | 12,836 | 67,924 |
| upper 95%-cl | 12,206 | 24,536 | 102,484 |

Figure 7: Results of regression shown in a table

3. ToxRat includes expert knowledge about statistics.

ToxRat takes care for selecting appropriate statistical methods. Thereby it focuses on methods used for statistical evaluation of single species biotests in ecotoxicology according to the OECD guidance document No. 54 (OECD 2006).

Individual biotest guidelines are updated from time to time and statistical methods might change over time. E.g., ECx calculation for growth rates obtained in a Duckweed growth inhibition test has been and is still performed frequently using linear regression, namely Probit analysis. However, in the mean time, more and more new and updated guidelines recommend to use non-linear regression rather than linear regression for metric data such as growth rates (e.g. OECD 201, 2011). This is due to several reasons, amongst others, normalizing of the treatment means by the mean control response is regarded to pose some principle problems (Green 2016). Even in the OECD guideline 221 (2006) for the Duckweed growth inhibition test, the non linear regression is mentioned as "preferred technique". The ToxRat software enables Probit-, Logit- and Weibull-analysis using linear regression, but following the recent state of the art, the default settings are preset to non linear regression using either 2,-3- or 4 parameter functions (Figure 8). Thereby, the default settings should be regarded as recommendations, which can be changed by the user, if desired.

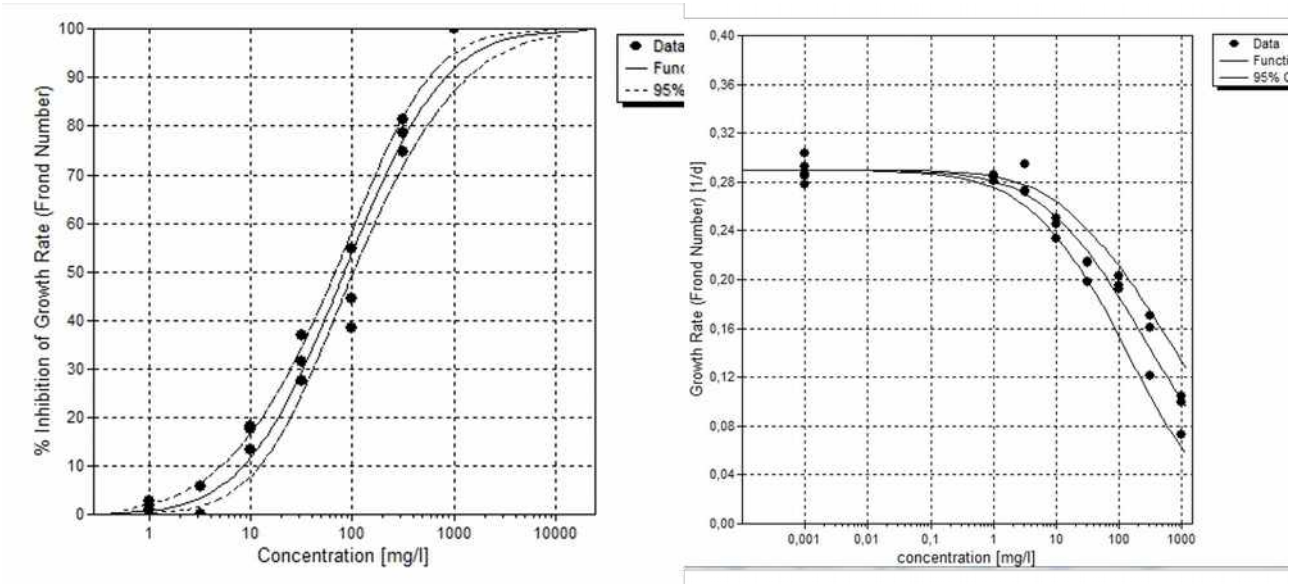



Figure 8: Graphical output of a linear (left) and a non linear (right) regression with growth rate data of variable frond number

The OECD test guideline No. 221 for the Duckweed growth inhibition test prescribes also the threshold concentrations, called NOEC, to be calculated as a toxic metric. However, no certain recommendation is given on which statistical tests should be used, except referring to the OECD document No. 54 (2006) for guidance. Also here, the user can rely on ToxRat: the software offers an automatic mode, running a complete sequence of pretests and selecting an appropriate final test depending on the results of the pretests. So, using default settings for evaluation in any case ends up with correct results. This enables evaluation of data sets even for not-statisticians and saves a lot of time with routine evaluation. Moreover, ToxRat is even recommended in an OECD guideline as appropriate software for statistical evaluation (OECD 243).

Though default settings will produce statistically correct results in either case, it goes without saying, that depending on the data, user settings might be required in order to further optimize evaluation. For this purpose, the advanced user can select individual settings, both for ECx calculation and NOEC determination (Figure 9).

## 4. Service and Support available

ToxRat is not simply just a software program. According to the principle that "a software is just as valuable as the support behind", the ToxRat team places the highest value on prompt and qualified customer service and effective, individual advice and assistance with software operation, data analysis, selecting an appropriate statistical method and interpreting the results.

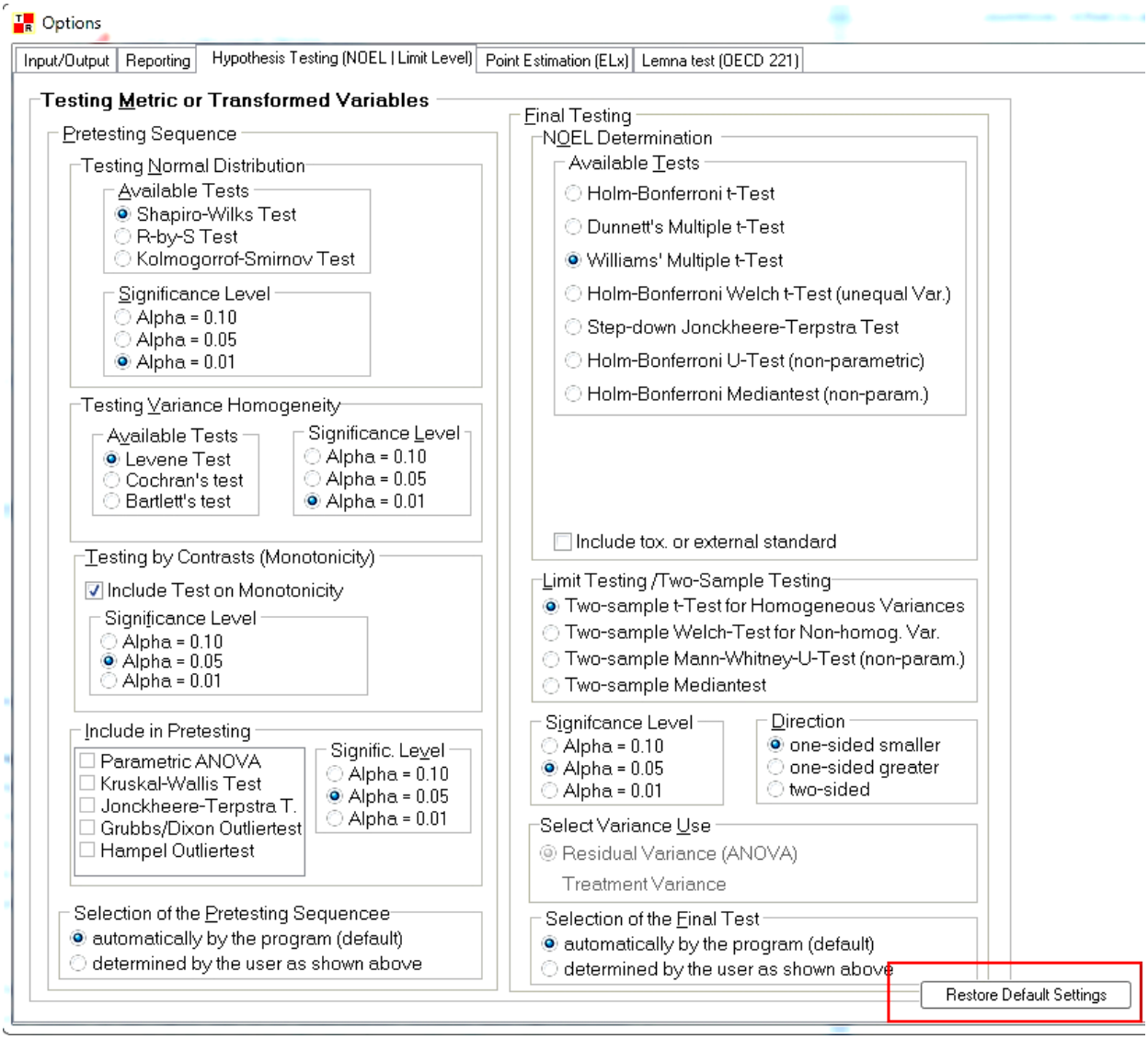



Figure 9: NOEC determination: Default settings available – user settings possible

## 5. Validation

The scope of delivery includes a validation document explaining all methods and mathematical formulae used by the program. All mathematical and statistical procedures are applied to standardized test data sets, and the results are verified in comparison to the results of the independent calculations (e.g. MS Excel) and data published in literature. The test data sets form part of the scope of delivery of the software, which means that the users can repeat this quality control step to verify that the software delivers the correct results as stated in the validation document, also in the user´s individual system configuration.

## Get your own impression

Trial versions are available at www.toxrat.com. They are free-of-charge and without any obligation. They enable to enter own data, to use all the settings, to carry out complete evaluations and to generate reports.

## References

Green, J.W. (2016): Normalizing data to Control Creates large Errors. SETAC Europe 26th Annual Meeting, Poster TH155

ISO 20079 (2005) Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) — Duckweed growth inhibition test

OECD (2006) Current approaches in the statistical analysis of ecotoxicity data: a guidance to application. Environment, Health and Safety Publications. Series on Testing and Assessment No. 54. OECD, Paris.

OECD 221 (2006) OECD Guidelines tor the testing of chemicals. *Lemna* sp. Growth Inhibition Test

OECD 201 (2011) OECD Guidelines tor the testing of chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test

OECD 243 (2016) OECD Guidelines tor the testing of chemicals. *Lymnaea stagnalis* Reproduction Test

# Student Spotlight: Barak Cohen

(barak330@gmail.com)

The complex interactions that take place inside and around the immediate surrounding of water plants attracted me since childhood. Observing different protozoa, insects, algae and plants drew me to spend a lot of time hovering around pools of fresh water. Little did I know that these amazing pools were just the tip of the iceberg.

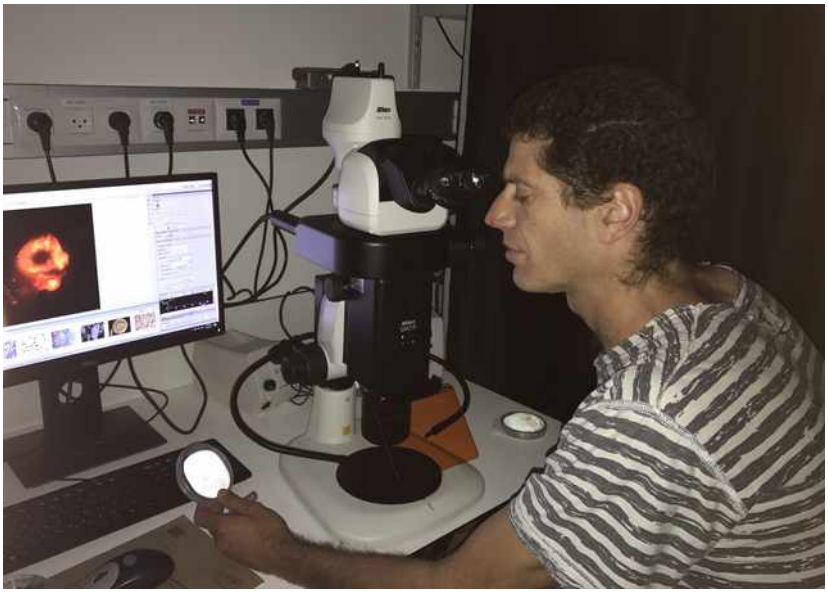
Later on in my life, I worked as a diving instructor and was exposed to the immense richness of life existing under the waters around the world. It was only natural for me to choose marine science for my first degree, surrounded by the students and teachers of Rupin College located on the shore of the Mediterranean Sea in Israel.

Among various courses there, I participated in oceanography research on board a research vessel, retrieving water samples, defining their properties and identifying different zooplankton communities.

Later on, I worked for a start-up company, growing a specific protozoan in high concentrations intended to be marketed as live food for the larvae of marine fish in marine agriculture. I was happy to get the opportunity to work in sea and lake research while situated on the shores of the Red sea.

My interest in duckweed started when I met Marvin Edelman four years ago at the Department of Plant & Environmental Sciences of the Weizmann Institute of Science. I started investigating the life cycle of a duckweed species under the influence of different chemical factors in the lab in order to better understand aspects such as the number of daughters each mother frond produced, the changes in rate of budding of daughter fronds during a single mature frond's life, and so on.

The time I spent looking at the plant through the binocular microscope allowed me to note subtle changes in phenotype and develop a simple approach to assess the general heath of a clonal population of the plants by the statistical distribution of it's different phenotypes.



A few years have passed since. During that time I learned how to maintain a collection of different duckweed strains and species in the lab, refine a protocol for starch quantification in duckweed and test the dynamics of starch accumulation and dry weight shifts

under different growth conditions. I was also involved in a project where plants were exposed to different mutagenic compounds, in order to produce stable mutated lines. The plants were screened using their altered phenotypes as an indication of mutagenesis. During this project, among other interesting mutations, I isolated a stable line that grew only when an organic carbon source was provided, and happily divided, green as ever, under water at the bottom of its growth vessel. These results were presented at the last duckweed conference that took place in Kerala, India in October 2017. It was an exciting experience for me to attend this event and present my work.

For the last year and a half, I have been involved in molecular duckweed research in Avi Levy's lab at the Weizmann Institute of Science as a part of my Master degree studies. With guidance by Ron Vunsh, I induced calli in several duckweed strains and regenerated them. I am now concentrating on transformation experiments and exploring new methods of increasing transient expression in a specific strain and silencing the expression of specific genes.

I anticipate that duckweed research will continue gaining in momentum as more researchers become deeply interested in this field. I feel highly satisfied working with a plant whose uniqueness is yet to be fully explored and provides me with freedom to test new ideas and research directions.

In addition to my lab research, I also participate in an exciting venture at an agrotech company focused on high-grade growth of duckweed as food for human consumption. As a part of the company team, I am involved in R&D at almost each step of the plant's growth.

I wish all of us an interesting future and exciting revelations in our duckweed research.

# From the database

## Biotechnology

### Catalytic production of levulinic acid and ethyl levulinate from uniconazole-induced duckweed (*Lemna minor*)

Liu, CG; Feng, QN; Yang, JR; Qi, XH (2018) BIORESOURCE TECHNOLOGY 255: 50-57

Duckweed (*Lemna minor*) with a high starch content of 50.4% was cultivated by uniconazole-induction method. The cultivated duckweed was used to produce value-added chemicals such as glucose, levulinic acid and formic acid in diluted HCl aqueous solution. A high glucose yield of 93.4% (471 g/kg based on loading duckweed mass) could be achieved at 180 °C in short reaction time, and the generated glucose was converted into levulinic acid and formic acid with yields of 52.0% and 34.1%, respectively, for 150 min, corresponding to 262 g/kg levulinic acid yield and 171 g/kg formic acid yield based on the mass of loading duckweed, respectively. Moreover, the duckweed was efficiently converted to ethyl levulinate with 55.2% yield (400.6 g/kg) at 200 °C in ethanol. This work provides a promising strategy for the production of value-added chemicals from phytoplankton that is able to purify the wastewater containing high content of P and N.

### Bioenergy potential of *Wolffia arrhiza* appraised through pyrolysis, kinetics, thermodynamics parameters and TG-FTIR-MS study of the evolved gases

Ahmad, MS; Mehmood, MA; Liu, CG; Tawab, A; Bai, FW; Sakdaronnarong, C; Xu, JR; Rahimuddin, SA; Gull, M (2018) BIORESOURCE TECHNOLOGY 253: 297-303

This study evaluated the bioenergy potential of *Wolffia arrhiza* via pyrolysis. The biomass was collected from the pond receiving city wastewater. Oven dried powdered biomass was exposed to thermal degradation at three heating rates (10, 30 and 50 degrees C $min^{-1}$) using Thermogravimetry-Differential Scanning Calorimetry analyzer in an inert environment. Data obtained were subjected to the isoconversional models of Kissenger-Akahira-Sunose (KSA) and Flynn-Wall-Ozawa (FWO) to elucidate the reaction chemistry. Kinetic parameters including, Ea (136-172 $kJmol^{-1}$) and Gibb's free energy (171 $kJmol^{-1}$) showed the remarkable bioenergy potential of the biomass. The average enthalpies indicated that the product formation is favored during pyrolysis. Advanced coupled TG-FTIR-MS analyses showed the evolved gases to contain the compounds containing C-O functional groups (aldehydes, ketones), aromatic and aliphatic hydrocarbons as major pyrolytic products. This low-cost abundant biomass may be used to produce energy and chemicals in a cost-efficient and environmentally friendly way.

## Aquatic weeds as the next generation feedstock for sustainable bioenergy production

Kaur, M; Kumar, M; Sachdeva, S; Puri, SK (2018) BIORESOURCE TECHNOLOGY 251: 390-402

Increasing oil prices and depletion of existing fossil fuel reserves, combined with the continuous rise in greenhouse gas emissions, have fostered the need to explore and develop new renewable bioenergy feedstocks that do not require arable land and freshwater resources. In this regard, prolific biomass growth of invasive aquatic weeds in wastewater has gained much attention in recent years in utilizing them as a potential feedstock for bioenergy production. Aquatic weeds have an exceptionally higher reproduction rates and are rich in cellulose and hemicellulose with a very low lignin content that makes them an efficient next generation biofuel crop. Considering their potential as an effective phytoremediators, this review presents a model of integrated aquatic biomass production, phytoremediation and bioenergy generation to reduce the land, fresh water and fertilizer usage for sustainable and economical bioenergy.

## Expression and Immunogenicity of M2e Peptide of Avian Influenza Virus H5N1 Fused to Ricin Toxin B Chain Produced in Duckweed Plants

Firsov, A; Tarasenko, I; Mitiouchkina, T; Shaloiko, L; Kozlov, O; Vinokurov, L; Rasskazova, E; Murashev, A; Vainstein, A; Dolgov, S (2018) FRONTIERS IN CHEMISTRY 6, Article Number: 22; DOI: 10.3389/fchem.2018.00022

The amino acid sequence of the extracellular domain of the virus-encoded M2 matrix protein (peptide M2e) is conserved among all subtypes of influenza A strains, enabling the development of a broad-range vaccine against them. We expressed M2e from avian influenza virus A/chicken/Kurgan/5/2005 (H5N1) in nuclear-transformed duckweed plants for further development of an avian influenza vaccine. The 30-amino acid N-terminal fragment of M2, including M2e (denoted M130), was selected for expression. The M2e DNA sequence fused in-frame to the 3' end of ricin toxin B chain (RTB) was cloned under control of the CaMV 35S promoter into pBI121. The resulting plasmid was used for duckweed transformation, and 23 independent transgenic duckweed lines were obtained. Asialofetuin-binding ELISA of protein samples from the transgenic plants using polyclonal anti-RTB antibodies confirmed the expression of the RTB-M130 fusion protein in 20 lines. Quantitative ELISA of crude protein extracts from these lines showed RTB-M130 accumulation ranging from 0.25-2.5 μg/g fresh weight (0.0006-0.01% of total soluble protein). Affinity chromatography with immobilized asialofetuin and western blot analysis of protein samples from the transgenic plants showed expression of fusion protein RTB-M130 in the aggregate form with a molecular mass of about 70 kDa. Mice were immunized orally with a preparation of total soluble protein from transgenic plants, receiving four doses of 7 mu g duckweed-derived RTB-M130 each, with no additional adjuvant. Specific IgG against M2e was detected in immunized mice, and the endpoint titer of nti-M2e IgG was 1,024. It was confirmed that oral immunization with RTB-M130 induces production of specific antibodies against peptide M2e, one of the most conserved antigens of the influenza virus. These results may provide further information for the development of a duckweed-based expression system to produce a broad-range edible vaccine against avian influenza.

## Hydroxycinnamoylmalated flavone C-glycosides from *Lemna japonica*

Bai, HH; Wang, NN; Mi, J; Yang, T; Fang, DM; Wu, LW; Zhao, H; Li, GY (2018) FITOTERAPIA 124: 211-216

Three previously undescribed flavone C-glycosides (1-3), along with seven known ones (4-10), were isolated and characterized from the smallest flowering aquatic plant, *Lemna japonica*. On the basis of spectroscopic analysis and alkaline hydrolysis, compounds 1-3 were identified to be luteolin 6-C-(2 ''-O-trans-caffeoyl-D-malate)-beta-glucoside (1), apigenin 6-C-(2 ''-O-trans-caffeoyl-D-malate)-beta-glucoside (2), and luteolin 6-C-(2 ''-O-trans-coumaroyl-D-malate)-beta-glucoside (3). Compounds 1-3 are characteristic of a trans-coumaroyl-D-malate or trans-caffeoyl-D-malate linked to C-2'' of the glucose, which was reported for the first time. Compounds 1-3 exhibited weak cytotoxicity against HepG-2, SW-620, and A-549 cell lines, with IC50 values between 42.5 and 19.2 μg/ml, and moderate antioxidant activity. Meanwhile compound 3 displayed moderate nematocidal activity with an EC50 value of 1.56 mg/ml.

## A simple modeling approach for characteristics analysis of hydrothermal liquefaction products from low-lipid aquatic plants

Guo, SQ; Dong, XY; Zhu, CX; Han, YY; Wang, ZZ (2017) APPLIED THERMAL ENGINEERING 125: 394-400

Hydrothermal liquefaction (HTL) product distributions highly depend on reaction temperature and holding time. This paper provided a new method to establish the isothermal model on the relationship between HTL product characteristics and severity factor (combined temperature and time). The results showed that the yields of the HTL bio-oil and aqueous-phase product presented strong correlations with reaction severity and could be modeled by Lorentz (or Gaussian) function, while the HTL solid char decreased gradually with increase of reaction severity, and could be modeled by the Dose-response function. All the mean adj. $R^2$ values of the models were greater than 0.89. The H/C, O/C ratio, dry ash-free carbon recovery (energy recovery) could be described by Bi-Dose-response, Dose-response, Dose response, and Lonrentz models, respectively. Low biomass/water ratio resulted in achieving the maximum bio-oil yield at low reaction severity. High bio-oil yield could be produced with low-ash feedstock. The maximum dry ash-free carbon recovery and energy recovery of Desmodesmus sp. (Dsp), Cyanobacteria sp. (Csp), Bacillariophyta sp. (Bsp), and Duckweed (Dw) dependeded less on the carbohydrate composition. The newly established models of the HTL yields provide a promising method for predicting the product characteristics, simulating, designing, and optimizing the HTL system.

# Ecology

## The invasive duckweed *Lemna minuta* Kunth displays a different light utilisation strategy than native *Lemna minor* Linnaeus

Paolacci, S; Harrison, S; Jansen, MAK (2018) AQUATIC BOTANY 146: 8-14

*Lemna minuta* Kunth is an invasive, alien duckweed that is present throughout much of Europe, where it competes with native congeneric *Lemna minor* Linnaeus. Previously, *L. minuta* was found to grow faster than *L. minor*. The aim of this study was to determine whether the rapid growth of

invasive *L. minuta* is based on differential light utilisation. For this purpose, the growth performance of *L. minuta* was compared with that of *L. minor* under a range of different light intensities. Both physiological and morphological parameters were determined. *L. minuta* showed a higher Relative Growth Rate (RGR) than *L. minor* when grown under medium and high intensities. Further analysis showed that, at high light intensities, *L. minuta* has a higher Net Assimilation Rate (NAR), and displays more photochemical quenching (qP) and a higher quantum yield (Y(II)) than *L. minor*. In contrast under low light intensities *L. minor* displayed a marginally higher RGR, due to a greater Leaf Area Ratio (LAR), and higher chlorophyll content than *L. minuta*. The results indicate two distinct light utilisation strategies, and reveal that the invasive species *L minuta* takes more advantage from high intensity light conditions. In turn, this may influence plant distribution and, consequently, ecosystem management by relevant authorities.

## Recovery of lake vegetation following reduced eutrophication and acidification

Baastrup-Spohr, L; Sand-Jensen, K; Olesen, SCH; Bruun, HH (2017) FRESHWATER BIOLOGY 62: 1847-1857

In recent decades, many aquatic ecosystems in Europe and North America have experienced reduced inputs of nutrients and acidifying substances because of improved sewage treatment and reduced emission of sulphur oxides. We evaluated the consequences of these efforts to changes in water chemistry, species richness and community composition of aquatic macrophytes in 56 lakes in Denmark around 1990 and again around 2010. Reductions in lake water concentrations of phosphorus and nitrogen were strongest in eutrophic and hypertrophic lakes, for example, lakes which had been heavily affected by domestic sewage. These changes translated into decreased algal biomass in the most eutrophied lakes. Oligo- and mesotrophic lakes did not change significantly in terms of nutrients or algal biomass. Water clarity increased across all lakes but not significantly in specific trophic lake groups. Alkalinity and pH increased significantly (up to 2pH-units) in low-alkaline lakes, while well-buffered high-alkaline lakes (>0.5meq/L) did not show any change. Macrophyte species richness per lake increased, on average, by 13% during the 20-year study period. The increase was strongest in species preferring nutrient-rich conditions and could be directly attributed to reductions in phytoplankton biomass in lakes of medium water clarity. The similarity among all lakes in terms of species composition increased over the study period. This development was closely related to higher average species richness and was mainly caused by recolonisation of lakes, recovering from past eutrophication, by relatively common species (e.g., *Lemna trisulca*, *Sparganium emersum* and *Potamogeton berchtoldii*). Higher pH in low-alkaline lakes was accompanied by a shift from acid-tolerant to more acid-sensitive species. Our results demonstrate that investment in pollution control has been successful in terms of markedly improving water quality of lakes and, with a time lag, macrophyte species richness. Although relatively common species have spread across lakes and resulted in homogenised macrophyte communities, continued efforts to reduce pollution could ensure the survival of rare specialist species and perhaps even increase their abundance in the future.

# Feed and Food

## Effect of dietary *Wolffia arrhiza* and *Spirulina platensis* on growth performance and pigmentation of Queen loach *Botia dario* (Hamilton, 1822)

Gogoi, S; Mandal, SC; Patel, AB (2018) AQUACULTURE NUTRITION 24: 285-291

The present experiment was conducted for 75days in triplicates groups in 18 aquaria of 50L each to study the effect of *Wolffia arrhiza* and *Spirulina platensis* on growth and pigmentation of *Botia dario*. Six isonitrogenous diets were prepared with 350g/kg crude protein (CP) level. Diet 1 (T1) was prepared without fortification of Spirulina and Wolffia. T2 diet was prepared with Spirulina as supplement of carotenoids. Similarly, T3, T4, T5 and T6 diets were prepared by substituting 25, 50, 75 and 100g/kg of CP from Spirulina with Wolffia, respectively. Significant differences ($p<.05$) in final mean weight, mean weight gain, body weight gain and specific growth rate were observed. 100 percent survivability was recorded in T4 and T5. Final carotenoids content (g/g) in skin (166.39 ± 2.71) and muscle (10.67 ± 0.32) was recorded highest in T5 and in whole fish in T6 (13.03 ± 0.95g/g). Redness, yellowness and whiteness found to have no significant differences ($p>.05$). Thus, it can be concluded that diet containing 100 g/kg Spirulina can be effective for better growth while diet containing 25 g/kg Spirulina and 150 g/kg Wolffia can be effective for higher survival and pigmentation in *Botia dario*.

# Molecular Biology

## Monitoring circadian rhythms of individual cells in plants

Muranaka, T; Oyama, T (2018) JOURNAL OF PLANT RESEARCH 131: 15-21

The circadian clock is an endogenous timing system based on the self-sustained oscillation in individual cells. These cellular circadian clocks compose a multicellular circadian system working at respective levels of tissue, organ, plant body. However, how numerous cellular clocks are coordinated within a plant has been unclear. There was little information about behavior of circadian clocks at a single-cell level due to the difficulties in monitoring circadian rhythms of individual cells in an intact plant. We developed a single-cell bioluminescence imaging system using duckweed as the plant material and succeeded in observing behavior of cellular clocks in intact plants for over a week. This imaging technique quantitatively revealed heterogeneous and independent manners of cellular clock behaviors. Furthermore, these quantitative analyses uncovered the local synchronization of cellular circadian rhythms that implied phase-attractive interactions between cellular clocks. The cell-to-cell interaction looked to be too weak to coordinate cellular clocks against their heterogeneity under constant conditions. On the other hand, under light-dark conditions, the heterogeneity of cellular clocks seemed to be corrected by cell-to-cell interactions so that cellular clocks showed a clear spatial pattern of phases at a whole plant level. Thus, it was suggested that the interactions between cellular clocks was an adaptive trait working under day-night cycles to coordinate cellular clocks in a plant body. These findings provide a novel perspective for understanding spatio-temporal architectures in the plant circadian system.

## Codon usage and codon pair patterns in non-grass monocot genomes

Mazumdar, P; Othman, RB; Mebus, K; Ramakrishnan, N; Harikrishna, JA (2017) ANNALS

Studies on codon usage in monocots have focused on grasses, and observed patterns of this taxon were generalized to all monocot species. Here, non-grass monocot species were analysed to investigate the differences between grass and non-grass monocots. First, studies of codon usage in monocots were reviewed. The current information was then extended regarding codon usage, as well as codon-pair context bias, using four completely sequenced non-grass monocot genomes (*Musa acuminata*, *Musa balbisiana*, *Phoenix dactylifera* and *Spirodela polyrhiza*) for which comparable transcriptome datasets are available. Measurements were taken regarding relative synonymous codon usage, effective number of codons, derived optimal codon and GC content and then the relationships investigated to infer the underlying evolutionary forces. The research identified optimal codons, rare codons and preferred codon-pair context in the nongrass monocot species studied. In contrast to the bimodal distribution of GC(3) (GC content in third codon position) in grasses, non-grass monocots showed a unimodal distribution. Disproportionate use of G and C (and of A and T) in two-and four-codon amino acids detected in the analysis rules out the mutational bias hypothesis as an explanation of genomic variation in GC content. There was found to be a positive relationship between CAI (codon adaptation index; predicts the level of expression of a gene) and GC(3). In addition, a strong correlation was observed between coding and genomic GC content and negative correlation of GC(3) with gene length, indicating a strong impact of GC-biased gene conversion (gBGC) in shaping codon usage and nucleotide composition in non-grass monocots. Optimal codons in these non-grass monocots show a preference for G/C in the third codon position. These results support the concept that codon usage and nucleotide composition in non-grass monocots are mainly driven by gBGC.

## The Chloroplast Genome of *Symplocarpus renifolius*: A Comparison of Chloroplast Genome Structure in Araceae

Choi, KS; Park, KT; Park, S (2017) GENES 8, Article Number: 324; DOI: 10.3390/genes8110324

*Symplocarpus renifolius* is a member of Araceae family that is extraordinarily diverse in appearance. Previous studies on chloroplast genomes in Araceae were focused on duckweeds (Lemnoideae) and root crops (Colocasia, commonly known as taro). Here, we determined the chloroplast genome of *Symplocarpus renifolius* and compared the factors, such as genes and inverted repeat (IR) junctions and performed phylogenetic analysis using other Araceae species. The chloroplast genome of *S. renifolius* is 158,521 bp and includes 113 genes. A comparison among the Araceae chloroplast genomes showed that infA in *Lemna, Spirodela, Wolffiella, Wolffia*, *Dieffenbachia* and *Colocasia* has been lost or has become a pseudogene and has only been retained in *Symplocarpus*. In the Araceae chloroplast DNA (cpDNA), psbZ is retained. However, psbZ duplication occurred in *Wolffia* species and tandem repeats were noted around the duplication regions. A comparison of the IR junction in Araceae species revealed the presence of ycf1 and rps15 in the small single copy region, whereas duckweed species contained ycf1 and rps15 in the IR region. The phylogenetic analyses of the chloroplast genomes revealed that Symplocarpus are a basal group and are sister to the other Araceae species. Consequently, infA deletion or pseudogene events in Araceae occurred after the divergence of *Symplocarpus* and aquatic plants (duckweeds) in Araceae and duplication events of rps15 and ycf1 occurred in the IR region.

# Physiology

## Aberrant clones: Birth order generates life history diversity in Greater Duckweed, *Spirodela polyrhiza*

Mejbel, HS; Simons, AM (2018) ECOLOGY AND EVOLUTION 8: 2021-2031

Environmental unpredictability is known to result in the evolution of bet-hedging traits. Variable dormancy enhances survival through harsh conditions, and is widely cited as a diversification bet-hedging trait. The floating aquatic plant, *Spirodela polyrhiza* (Greater Duckweed), provides an opportunity to study diversification because although partially reliable seasonal cues exist, its growing season is subject to an unpredictable and literally hard termination when the surface water freezes, and overwinter survival depends on a switch from production of normal daughter fronds to production of dense, sinking turions prior to freeze-over. The problem for *S. polyrhiza* is that diversified dormancy behavior must be generated among clonally produced, genetically identical offspring. Variation in phenology has been observed in the field, but its sources are unknown. Here, we investigate sources of phenological variation in turion production, and test the hypothesis that diversification in turion phenology is generated within genetic lineages through effects of parental birth order. As expected, phenotypic plasticity to temperature is expressed along a thermal gradient; more interestingly, parental birth order was found to have a significant and strong effect on turion phenology: Turions are produced earlier by late birth-order parents. These results hold regardless of whether turion phenology is measured as first turion birth order, time to first turion, or turion frequency. This study addresses a question of current interest on potential mechanisms generating diversification, and suggests that consistent phenotypic differences across birth orders generate life history variation.

## Offspring of older parents are smaller-but no less bilaterally symmetrical-than offspring of younger parents in the aquatic plant *Lemna turionifera*

Ankutowicz, EJ; Laird, RA (2018) ECOLOGY AND EVOLUTION 8: 679-687

Offspring quality decreases with parental age in many taxa, with offspring of older parents exhibiting reduced life span, reproductive capacity, and fitness, compared to offspring of younger parents. These parental age effects, whose consequences arise in the next generation, can be considered as manifestations of parental senescence, in addition to the more familiar age-related declines in parent-generation survival and reproduction. Parental age effects are important because they may have feedback effects on the evolution of demographic trajectories and longevity. In addition to altering the timing of offspring life-history milestones, parental age effects can also have a negative impact on offspring size, with offspring of older parents being smaller than offspring of younger parents. Here, we consider the effects of advancing parental age on a different aspect of offspring morphology, body symmetry. In this study, we followed all 403 offspring of 30 parents of a bilaterally symmetrical, clonally reproducing aquatic plant species, *Lemna turionifera*, to test the hypothesis that successive offspring become less symmetrical as their parent ages, using the Continuous Symmetry Measure as an index. Although successive offspring of aging parents older than one week became smaller and smaller, we found scant evidence for any reduction in bilateral symmetry.

### Allelopathic effects of exogenous phenylalanine: a comparison of four monocot species

Evans, BR; Bali, G; Ragauskas, A; Shah, R; O'Neill, H; Howard, C; Lavenhouse, F; Ramirez, D; Weston, K; Ramey, Ket al. (2017) PLANTA 246: 673-685

Exogenous phenylalanine stunted annual ryegrass but not switchgrass or winter grain rye, with deuterium incorporation up to 3% from phenyalanine-d (8). Toxicity to duckweed varied with illumination intensity and glucose uptake. Isotopic labeling of biomolecules through biosynthesis from deuterated precursors has successfully been employed for both structural studies and metabolic analysis. Phenylalanine is the precursor of many products synthesized by plants, including the monolignols used for synthesis of lignin. Possible allelochemical effects of phenylalanine have not been reported, although its deamination product cinnamic acid is known to have deleterious effects on root elongation and growth of several plant species. The effects of phenylalanine and its deuterated analog phenylalanine-d (8) added to growth media were studied for annual ryegrass (*Lolium multiflorum*), winter grain rye (*Secale cereale*), and switchgrass (*Panicum virgatum*) cultivated under hydroponic conditions. Growth of annual ryegrass was inhibited by phenylalanine while switchgrass and rye were not significantly affected. Growth was less affected by deuterated phenylalanine-d (8) than by its protiated counterpart, which may be a typical deuterium kinetic isotope effect resulting in slower enzymatic reaction rates. Deuterium incorporation levels of 2-3% were achieved in biomass of switchgrass and annual ryegrass. Both protiated and deuterated phenylalanine were moderately toxic (IC25 values 0.6 and 0.8 mM, respectively) to duckweed (*Lemna minor*) grown using a 12 h diurnal cycle under photoautotrophic conditions. A significant increase in toxicity, greater for the deuterated form, was noted when duckweed was grown under higher intensity, full spectrum illumination with a metal halide lamp compared to fluorescent plant growth lamps emitting in the blue and red spectral regions. Supplementation with glucose increased toxicity of phenylalanine consistent with synergy between hexose and amino acid uptake that has been reported for duckweed.

## Phytoremediation

### Effect of zinc ions on nutrient removal and growth of *Lemna aequinoctialis* from anaerobically digested swine wastewater

Zhou, Q; Lin, Y; Li, X; Yang, CP; Han, ZF; Zeng, GM; Lu, L; He, SY (2018) BIORESOURCE TECHNOLOGY 249: 457-463

The effect of $Zn^{2+}$ on ammonium and phosphorous removal and duckweed growth was evaluated for treatment of anaerobically digested swine wastewater (ADSW) at various initial $Zn^{2+}$ concentrations ranging from 1.0 to 15 mg/L. *Lemna aequinoctialis* taken from a local pond was selected for the treatment, and its fresh weight and contents of proteins, photosynthetic pigments, and vitamin E were examined. Results showed that the optimal $Zn^{2+}$ concentration was 5.0 mg/L for $NH_3$-N and TP removal, the duckweed growth, and the accumulation of proteins in the duckweed. A maximum content of photosynthetic pigments increased with the increase of initial $Zn^{2+}$ concentration, and it arrived earlier for a higher concentration of $Zn^{2+}$. Vitamin E content in the duckweed reached 4.5 mg/kg at 15 mg/L $Zn^{2+}$ in 12-day cultivation, which showed the potential for producing and harvesting a high value-added product of vitamin E by culturing duckweed in ADSW.

## The accumulation of La, Ce and Y by *Lemna minor* and *Lemna gibba* in the Keban gallery water, Elazig Turkey

Sasmaz, M; Obek, E; Sasmaz, A (2018) WATER AND ENVIRONMENT JOURNAL 32: 75-83

This study investigated the phytoremediation ability of *Lemna minor* and *Lemna gibba* to accumulate lanthanum, cerium and yttrium from gallery water polluted by metals. *L. minor* and *L. gibba* were settled in the mining water and adapted to separate reactors. During the experiment, the water and plant samples were daily taken and the temperature, electric conductivity and pH of the water were daily measured in situ. These plants were firstly washed, dried in and then ashed at 300 °C for 24 h in an oven. Both water and ashed plant samples were measured by ICP-MS to detect the concentrations of lanthanum (La), cerium (Ce) and yttrium (Y). Although these elements are at low concentrations in gallery water, they were accumulated at the highest levels in *L. gibba* and in *L. minor*. This study showed that both plants have high ability to remove lanthanum, cerium and yttrium in gallery water polluted by different elements.

## Fate of estrone in laboratory-scale constructed wetlands

Hakk, H; Sikora, L; Casey, FXM (2018) ECOLOGICAL ENGINEERING 111: 60-68

A horizontal, subsurface, laboratory-scale constructed wetland (CW) consisting of four cells in series was used to determine the attenuation of the steroid hormone estrone (E1) present in animal wastewater. Liquid swine manure diluted 1: 80 with farm pond water and dosed with [C-14] E1 flowed through the series of cells containing floating vegetation (duckweed, family Lemnaceae) and a sand: gravel sediment layer. The aqueous layer within each cell was sampled across time, and at 168 h duckweed and sediment were sampled for E1 and/ or its metabolites. Control and Blank systems consisted of single cells with no vegetation or no vegetation/ sediment, respectively. Only 5% of the dose was detected in the effluent at 168 h. With the use of mass spectrometry and thin layer chromatography, it was determined that E1 was metabolized into estriol (E3) and to polar metabolites. The largest compartment for [C-14] was duckweed (42.1% of administered dose) at 168 h followed by sediment (17.3%) and the liquid layer (15.1% of the dose). Most of the vegetative removal of E1 occurred in the first cell, and thereafter, sediment: liquid layer interactions governed E1 movement suggesting particle-bound transport on either colloids or dissolved organic matter. Horizontal, subsurface flow CW were able to remove approximately 95% of the influent E1, and demonstrated the importance of vegetative matter in removal of this potent steroid hormone.

## A combination method based on chitosan adsorption and duckweed (*Lemna gibba* L.) phytoremediation for boron (B) removal from drinking water

Turker, OC; Baran, T (2018) INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 20:

The metalloid boron (B) and its compounds widely exist in the environment, and boron can have hazardous effects on plants, animals, and human beings when it is found in high concentrations in water bodies. It is difficult and costly to remove B with conventional treatment methods from drinking water. Therefore, alternative and cost-effective treatment techniques are necessary. In this study, for the first time, a novel and environmentally friendly method based on the phytoremediation ability of chitosan and duckweed (*Lemna gibba* L.) combination was evaluated for B removal from drinking water. Our results from batch adsorption experiment indicated that the highest B uptake capacity of chitosan bead was found as 3.18 mg/g, and we determined the optimal B sorption

occurs at pH value of 7. The Langmuir isotherm and pseudo-second-order kinetic model better fitted the equilibrium obtained for B removal. B in drinking water could be reduced to less than 2.4mg $L^{-1}$ when 0.05 g of plant-based chitosan beads and 12 *L. gibba* fronds were used in the 4-day treatment period.

## Bioremediation of heavy metal contaminated medium using *Lemna minor*, *Daphnia magna* and their consortium

Fikirdesici-Ergen, S; Ucuncu-Tunca, E; Kaya, M; Tunca, E (2018) CHEMISTRY AND ECOLOGY 34: 43-55

Single, dual and triple mixtures of totally seven different mixture combinations of the metals Al, Ba and Fe were examined in *Lemna minor* (L) culture, *Daphnia magna* (D) culture and in a consortium culture consisting of *L. minor* and *D. magna*. In this study: (a) differences in removed metal proportions at the end of 24 and 48h, (b) differences in removed metal amounts due to cultures, (c) differences in the removed proportions of a metal in distinct mediums and (d) removal correlation of the metals due to cultures were investigated. The study results showed that among the metals Al, Ba and Fe, Al has the most toxic effect on organisms involved in this study. Although similar toxicity results of Al and Fe on test groups were observed, Ba gave different toxicity results on test groups. An overview of the bioremediation results indicates that *L. minor* removes the metals Al and Fe more successfully than Ba. Different mixture combinations of metals performed dissimilar removal results in the same cultures. According to the correlations analysis for the metals Ba and Fe, a high correlation was recorded between the consortium group and test groups containing only *D. magna*, r=0.88, r=0.91, respectively.

## Treatment of synthetic textile wastewater containing dye mixtures with microcosms

Yaseen, DA; Scholz, M (2018) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 25: 1980-1997

Conference: International Conference on Conservation Agriculture and Sustainable Land Use (CASLU), Location: Budapest, HUNGARY Date: MAY 31-JUN 02, 2016

The aim was to assess the ability of microcosms (laboratory-scale shallow ponds) as a post polishing stage for the remediation of artificial textile wastewater comprising two commercial dyes (basic red 46 (BR46) and reactive blue 198 (RB198)) as a mixture. The objectives were to evaluate the impact of *Lemna minor* L. (common duckweed) on the water quality outflows; the elimination of dye mixtures, organic matter, and nutrients; and the impact of synthetic textile wastewater comprising dye mixtures on the *L. minor* plant growth. Three mixtures were prepared providing a total dye concentration of 10 mg/l. Findings showed that the planted simulated ponds possess a significant ($p < 0.05$) potential for improving the outflow characteristics and eliminate dyes, ammonium-nitrogen ($NH_4$-N), and nitrate-nitrogen ($NO_3$-N) in all mixtures compared with the corresponding unplanted ponds. The removal of mixed dyes in planted ponds was mainly due to phyto-transformation and adsorption of BR46 with complete aromatic amine mineralisation. For ponds containing 2 mg/l of RB198 and 8 mg/l of BR46, removals were around 53%, which was significantly higher than those for other mixtures: 5 mg/l of RB198 and 5 mg/l of BR46 and 8 mg/l of RB198 and 2 mg/l of BR46 achieved only 41 and 26% removals, respectively. Dye mixtures stopped the growth of *L. minor*, and the presence of artificial wastewater reduced their development.

## Simultaneous phytoremediation of chromium and phenol by *Lemna minuta* Kunth: a promising biotechnological tool

Paisio, CE; Fernandez, M; Gonzalez, PS; Talano, MA; Medina, MI; Agostini, E (2018) INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCE AND TECHNOLOGY 15: 37-48

The aim of this work was to evaluate the usefulness of *Lemna minuta* Kunth for the simultaneous removal of Cr(VI) and phenol. The impact of these contaminants on plant growth and some biochemical processes have also been discussed for a better understanding and utilization of this species in the field of phytoremediation. The optimal growth conditions and plant tolerance to Cr(VI) and/or phenol as well as removal were determined. Plants exposed to Cr(VI) and phenol were able to efficiently grow and remove both contaminants at high concentrations (up to 2.5 and 250 mg/L, respectively) after 21 days, indicating that they were resistant to mixed contamination. There were no significant differences between chlorophyll, carotene and malondialdehyde content of treated plants with respect to the controls, which would be due to an efficient antioxidant response. *L. minuta* showed a higher biomass than control without contaminant when was exposed to low concentrations of Cr(VI), suggesting an hormesis effect. The main removal process involved in chromium phytoremediation would be sorption or accumulation in the biomass. Moreover, our results suggest that phenol could be used as a donor of carbon and energy by these plants. These findings demonstrated that *Lemna minuta* Kunth might be suitable for treatment of different solutions contaminated with Cr(VI) and phenol, showing a high potential to be used in the treatment of effluents containing mixed contamination.

## Arsenic-hyperaccumulation and antioxidant system in the aquatic macrophyte *Spirodela intermedia* W. Koch (Lemnaceae)

da-Silva, CJ; Canatto, RA; Cardoso, AA; Ribeiro, C; Oliveira, JA (2017) THEORETICAL AND EXPERIMENTAL PLANT PHYSIOLOGY 29: 203-213

Phytoremediation is a promising and efficient alternative for removing arsenic (As) from contaminated soil and water. Plants used in phytoremediation should be able to remove the pollutant and tolerate the damage caused by it. Here, we investigated the potential of an aquatic macrophyte, *Spirodela intermedia* W. Koch (Lemnaceae), to accumulate As with minimal harmful effects. The As accumulation by *S. intermedia* increased with the increase of metalloid concentration in the solution until 2.0 mg As $L^{-1}$. The increase of As in the plants boosted the production of reactive oxygen species (ROS), such as $H_2O_2$ and $O_2^-$, which increased the amount of malondialdehyde. The activity of antioxidant enzymes, i.e. superoxide dismutase, peroxidases and glutathione reductase, as well as the concentration of a non-enzymatic antioxidant, i.e. anthocyanins, were triggered in response to ROS accumulation. Such antioxidant system was able to control the overproduction of $H_2O_2$, but not $O_2^-$. Thus, the damage observed in root tips of plants treated with 2.0 mg As $L^{-1}$ can be attributed to high and uncontrolled concentration of $O_2^-$. In addition to ROS control, *S. intermedia* tolerance to As was demonstrated to be associated with decreases in sulfur concentration, and maintenance of phosphorus concentration on the root surface. Overall, our results suggest that *S. intermedia* is a potential species for phytoremediation of As-contaminated aquatic environments because it absorbs large amounts of As, and presents important mechanisms of tolerance to this pollutant. Eight complete duckweeds chloroplast genomes were assembled, with lengths ranging from 165,775 bp to 171,152 bp, and each contains 80 protein-coding sequences, four rRNAs, 30 tRNAs and two pseudogenes. The identity of *L. punctata* strain ZH0202 chloroplast genomes assembled through two methods was 100%, and their sequences and lengths were completely identical. The chloroplast genome comparison demonstrated that the differences in chloroplast

genome sizes among the Lemnoideae primarily resulted from variation in non-coding regions, especially from repeat sequence variation. The phylogenetic analysis demonstrated that the different genera of Lemnoideae are derived from each other in the following order: *Spirodela, Landoltia, Lemna, Wolffiella,* and *Wolffia*.

## Assessing the effectiveness of pollutant removal by macrophytes in a floating wetland for wastewater treatment

Prajapati, M; van Bruggen, JJA; Dalu, T; Malla, R (2017) APPLIED WATER SCIENCE 7: 4801-4809

The study aimed to evaluate the removal of pollutants by floating treatment wetlands (FTWs) using an edible floating plant, and emergent macrophytes. All experiments were performed under ambient conditions. Physico-chemical parameters were measured, along with microbiological analysis of biofilm within the roots, water column, and sludge and gravel zone. Nitrification and denitrification rates were high in the water zone of *Azolla filiculoides*, *Lemna minor*, *Lactuca sativa*, *P. stratiotes*, and *Phragmites australis*. Phosphate removal efficiencies were 23, 10, and 15% for the free-floating hydrophytes, emergent macrophytes, and control and edible plants, respectively. The microbial community was relatively more active in the root zone compared to other zones. *Pistia stratiotes* was found to be the efficient in ammonium (70%) and total nitrogen (59%) removal. Pistia stratiotes also showed the highest microbial activity of 1306 mg $day^{-1}$, which was 62% of the total volume. Microbial activity was found in the water zone of all FTWs expect for *P. australis*. The use of *P. stratiotes* and the edible plant *L. sativa* could be a potential option to treat domestic wastewater due to relatively high nutrient and organic matter removal efficiency.

## Removal of nutrients, trace organic contaminants, and bacterial indicator organisms in a demonstration-scale unit process open-water treatment wetland

Bear, SE; Nguyen, MT; Jasper, JT; Nygren, S; Nelson, KL; Sedlak, DL (2017) ECOLOGICAL ENGINEERING 109: 76-83

A demonstration-scale unit process open-water wetland system was built to treat water from an effluent-dominated river (i.e., a river in which the flow consisted almost entirely of municipal wastewater effluent from May to October). Monitoring of the system over a two-year period indicated effective removal of nitrate, with concentrations decreasing by over 90% during summer. The temperature-independent areal first-order nitrate removal rate constant, k(20), ranged from 61.7 to 68.1 m $yr^{-1}$ after the microbial community was established, which is significantly higher than values typically observed in full-scale surface flow wetlands. The beta-adrenergic blockers, atenolol and propranolol, as well as the antiviral drug, acyclovir, were removed by photolysis and biotransformation in the wetland biomat, whereas the antiepileptic drug, carbamazepine, exhibited little removal. The bacterial indicators *E. coli* and enterococci decreased substantially during summer, mainly through sunlight exposure. Models of contaminant removal based upon measured flow rates and performance data collected at a similar pilot-scale system agreed well with measured data for nitrate and the trace organic contaminants. The model accurately predicted removal of enterococci but systematically over-predicted the removal of *E. coli*. During the two-year study period, routine maintenance was necessary to prevent colonization of the water surface with duckweed (*Lemna* spp.). Unit process open-water (UPOW) wetlands may offer a low-cost means of improving water quality in natural treatment systems that can be integrated with conventional surface-flow wetlands and other managed natural systems. The quantitative models of contaminant

removal described in this study can be used to design natural treatment systems that balance the needs for local water quality requirements, available land and site-specific requirements.

## Combination of aquatic species and safeners improves the remediation of copper polluted water

Panfili, I; Bartucca, ML; Ballerini, E; Del Buono, D (2017) SCIENCE OF THE TOTAL ENVIRONMENT 601: 1263-1270

In the last decades, many anthropogenic activities have resulted in heavy metal contamination of fresh waters and surrounding environments. This poses serious threats to human health. Phytoremediation is a cost-effective technology which is useful for remediating polluted soils and water. Recently, the use of aquatic free-floating plants has been proposed to remediate polluted water. In this context, a study on the capacity of two aquatic plants, *Lemna minor* (duckweed) and *Salvinia auriculata* (salvinia), to remediate $Cu^{+2}$ (Cu) polluted water was carried out. Initially, the species were exposed to different copper concentrations (1, 5, 10, 20 and 50 μmol $L^{-1}$) in order to assess $Cu^{+2}$ toxicity to the plants. In addition, plants were treated with two safeners (benoxacor and dichlormid), with the aimof pointing out any safening effect of these compounds on the aquatic species. Toxicity tests showed that safened plants had a greater Cu resistance, especially at the higher Cu doses. Finally, unsafened and safened plants were tested in the decontamination of water polluted by copper (1.2 mg $L^{-1}$). In general, duckweed removed higher amounts of Cu from polluted water than salvinia, and, surprisingly, for both the species the safeners significantly increased the plants' capacity to remove the metal from the polluted waters. Lastly, an HPLC-based method was developed and standardized to monitor the residual amounts of the two safeners in the water. While dichlormid was completely absorbed by duckweed within few days after the treatments, some residual amounts of both safeners were found in Salvinia vegetated water after two weeks. In conclusion, the results of this research show that the use of aquatic species in combination with safeners is an attractive and reliable tool to make plants more effective in phytoremediation of water polluted with metals (or other toxic compounds).

## Diisopropyldithiophosphoric acid-impregnated macroporous non-ionogenic styrene-divinylbenzene polymeric sorbent (Porolas) for effective copper extraction

Daminova, SS; Kadirova, ZC; Sharipov, KT; Stoyko, OV; Chepulsky, SA; Adewuyi, A; Hojamberdiev, M (2017) JOURNAL OF INDUSTRIAL AND ENGINEERING CHEMISTRY 55: 204-214

The extraction and sorption of copper from wastewaters can be improved by applying solvent impregnated resins (SIRs) with chelating organic extractant. The SIRs are capable of increasing the sorption capacity of expensive ion-exchange resin in order to enhance the performance of traditional liquid-liquid extraction by organic solvents thus reducing copper pollution in water. In this present study, macroporous non-ionogenic styrene-divinylbenzene polymeric sorbent (Porolas) matrix was impregnated with diisopropyldithiophosphoric acid (DIPDTP) to enhance $Cu^{2+}$ sorption in aqueous system. The influence of pore-filling degree (0-100%) by DIPDTP on copper sorption from aqueous chloride solution (pH = 1.95-10.7) was also evaluated. Higher degree of pore-filling by DIPDTP led to a significant decrease of the specific surface area (S-BET) of the DIPDTP-Porolas sorbents, confirming an effective impregnation. The $Cu^{2+}$ adsorption isotherm fitted well for Freundlich isotherm, and the maximum K-F (8.45 g $L^{-1}$) was obtained for 50% DIPDTP-Porolas with 97-99% $Cu^{2+}$ uptake due to the formation of $Cu^{2+}$-complexes, CuClL2-t(HL)(q). The $Cu^{2+}$ adsorption kinetic followed pseudo-second-order kinetic model at pH = 4-5 while the *Lemna minor* ecotoxicity test

revealed absence of toxic secondary pollution of wastewater. The DIPDTP-impregnated Porolas was found to be an efficient sorbent for simple, safe, and environment-friendly extraction of Cu2+ from industrial wastewaters using small amount of organic solvent.

## Removal mechanisms of benzotriazoles in duckweed *Lemna minor* wastewater treatment systems

Gatidou, G; Oursouzidou, M; Stefanatou, A; Stasinakis, AS (2017) SCIENCE OF THE TOTAL ENVIRONMENT 596: 12-17

The fate of five benzotriazoles (1H-benzotriazole, BTR; 4-methyl-1H-benzotriazole, 4TTR; 5-methyl-1H-benzotriazole, 5TTR; xylytriazole, XTR and 5-chlorobenzotriazole, CBTR) was studied in batch and continuous-flow *Lemna minor* systems and the role of different mechanisms on their removal was evaluated. Single and joint toxicity experiments were initially conducted using the Organization for Economic Co-operation and Development (OECD) protocol 221 and no inhibition on specific growth rate of *Lemna minor* was observed for concentrations up to 200 μg $L^{-1}$. All tested substances were significantly removed in batch experiments with *Lemna minor*. Excepting 4TTR, full elimination of CBTR, XTR, 5TTR and BTR was observed up to the end of these experiments (36 d), while the half-life values ranged between 1.6 ± 0.3 d (CBTR) and 25 ± 3.6 d (4-TTR). Calculation of kinetic constants for hydrolysis, photodegradation, and plant uptake revealed that for all BTRs the kinetic constants of plant uptake were by far higher comparing to those of the other mechanisms, reaching 0.394 ± 0.161 $d^{-1}$ for CBTR. The operation of a continuous-flow *Lemna minor* system consisted of three mini ponds and a total hydraulic residence time of 8.3 d showed sufficient removal for most target substances, ranging between 26% (4TTR) and 72% (CBTR). Application of a model for describing micropollutants removal in the examined system showed that plant uptake was the major mechanism governing BTRs removal in *Lemna minor* systems.

## Duckweed utilization for fresh water conservation (management) in recirculated aquaculture systems

Popa, R; Moga, IC; Rissdorfer, M; Ilis, MLG; Petrescu, G; Craciun, N; Matache, MG; Covaliu, CI; Stoian, G (2017) INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE 8: 715-

The paper aims at presenting key aspects of the use of duckweed in the industry applications and a concept for using duckweed bio filters in aquaculture systems. Duckweed is a plant adapted to aquatic systems and considered to be one of the smallest plants in the world, with a diameter of 1-15 mm. Although it is a small plant, its properties make it suitable for industrial use (production of bio fuel, aquaponic, food source, waste water treatment). Several aspects of the duckweed crop management are mentioned in the paper. It has been shown that certain duckweed species can successfully lower the Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), total nitrogen (TN), total phosphorus (TP) and orthophosphate (OP). The authors propose a new waste water treatment technology with duckweed tanks for recirculated aquaculture systems.

## Treatment performance and macrophytes growth in a restored hybrid constructed wetland for municipal wastewater treatment

Barco, A; Borin, M (2017) ECOLOGICAL ENGINEERING 107: 160-171

This work evaluated depuration performance and macrophyte plants growth in a full-scale hybrid constructed wetland (H-CW) (1000 population equivalent) treating municipal wastewater. The plant

was activated in June 2010 after a restoration of an existent H-CW composed of a horizontal subsurface flow (HSSF) bed vegetated with evergreen xerophile species (*Prunus laurocerasus*, *Pittosporum spp*., *Elaeagnus angustifolia*), connected with a sedimentation pond vegetated with *Lemna* spp. To enhance the depuration performances, in both sections of the plant the original vegetation was removed and the HSSF bed was vegetated with *Phragmites australis*, whereas in the pond, a floating treatment wetland vegetated with *Iris pseudacorus* was installed. The depuration performance in treating total nitrogen (TN), nitrate nitrogen ($NO_3$-N), ammonia nitrogen ($NH_4$-N), total phosphorus (TP), orthophosphate ($PO_4$-P) and chemical oxygen demand (COD) was weekly monitored between August 2010 and August 2011. Results indicated median concentration abatements for the entire system of 74.3% for TN, 62.1% for $NH_4$-N, 77.7% for $NO_3$-N, 29.6% for TP, 37.4% for $PO_4$-P, and 46.7% for COD. In addition, at the end of the first growing season (June 2010-November 2010), good adaption to the system was detected for both *P. australis* and pseudacorus, which produced respectively 3.9 ± 2.2 kg $m^{-2}$ and 3.7 ± 1.0 kg $m^{-2}$ of above-ground dry biomass, with average above-ground N uptakes of 62.4 ± 35.6 g $m^{-2}$ and 69.8 ± 19.0 g $m^{-2}$ and average above-ground P uptakes of 4.6 ± 2.6 g $m^{-2}$ and 7.8 ± 2.1 g $m^{-2}$. The results indicate that a H-CW composed of a sequence of HSSF bed and FTW represents an effective application for abating TN, $NH_4$-N, $NO_3$-N and COD concentrations from municipal wastewater.

# Phytotoxicity

## Wood ash residue causes a mixture of growth promotion and toxicity in *Lemna minor*

Jagodzinski LS; O'Donoghue MT; Heffernan LB; van Pelt FNAM.; O'Halloran J; Jansen MAK (2018) SCIENCE OF THE TOTAL ENVIRONMENT 625: 667-676

The use of wood as a sustainable biofuel results in the generation of residual wood ash. The ash contains high amounts of plant macronutrients such as phosphorus, potassium, calcium as well as several micronutrients. To explore the potential use of wood ash as a fertiliser, the growth enhancing properties of Sitka spruce (*Picea sitchensis* Bong.) wood ash were contrasted with the potential toxic action, using common duckweed (*Lemna minor* L.) as a model test species. The growth of *L. minor* exposed to wood bottom and fly ash solids and corresponding leachates was assessed in ultra-oligotrophic and eutrophic media. Ash solids and leachates were also tested as neutralized preparations. Suspended ash solids promoted *L. minor* growth up to concentrations of 2.5-5 g/L. Leachates promoted growth up to 10 g ash equivalents per litre, but for bottom ash only. Beneficial effects of wood ash were most pronounced on ultra-oligotrophic medium. However, on such nutrient-deficient medium severe inhibition of *L. minor* biomass and frond growth was observed at relatively low concentrations of fly ash (EC50 = 14 g/L). On standard, eutrophic medium, higher concentrations of fly ash (EC50 = 21 g/L), or neutralized fly ash (EC50 = 37 g/L) were required to impede growth. Bottom ash, or neutralized bottom ash retarded growth at concentrations of 51 g/L and 74 g/L (EC50), respectively, in eutrophic medium. It appears that phytotoxicity is due to the elemental composition of the ash, its alkaline character, and possible interactions between these two properties. Growth promotion was due to the substantial content of plant nutrients. This study underlines the importance of the receiving environment (nutrient status and pH) in determining the balance between toxicity and growth promotion, and shows that the margin between growth promoting and toxicity inducing concentrations can be enlarged through ash neutralization.

## Effects of five sulphonamides on duckweed (*Lemna minor*) after prolonged exposure time and their dependency on photoradiation

Bialk-Bielinska, A; Matzke, M; Caban, M; Stolte, S; Kumirska, J; Stepnowski, P (2018) SCIENCE OF THE TOTAL ENVIRONMENT 618: 952-960

Sulphonamides (SAs) are one of the most commonly used veterinary drugs and therefore their residues are regularly found in the environment. So far scientific attention has mostly been paid to the evaluation of their acute ecotoxicological effects with data on long-term effects for non-target organisms still largely missing. Therefore, the main aim of this study was to evaluate the potential toxicities of five sulphonamides to duckweed (*Lemna minor*) after prolonged exposure time (14 days). To elucidate whether their phytotoxic effects result from potential photodegradation products, the toxicity of standard solutions of selected sulphonamides was also investigated in a standard 7-day test but after irradiation (by keeping them under the test conditions) for the selected time (after 7 and 14 days). The ecotoxicological tests were accompanied by chemical analyses to be able to link the observed effects to the concentrations and nature of the exposed compounds. The results showed a shift in the toxicity of SAs: a strong decrease in toxicity for the two most toxic sulphonamides (sulphamethoxazole and sulphadimethoxine) and a slight increase in toxicity for three other SAs (sulphadimidine, sulphathiazole, sulphamerazine) in the prolonged test. However, a decrease in the toxicity and concentration of all the SAs was observed when stock solutions were irradiated prior to the toxicity experiment, which suggests that the observed effects towards *L. minor* of five SAs in the prolonged test cannot be directly associated with the degradation of these compounds under the test conditions but with their different mode of toxic action towards these organisms.

## Phytotoxicity of silver nanoparticles to *Lemna minor*: Surface coating and exposure period-related effects

Pereira, SPP; Jesus, F; Aguiar, S; de Oliveira, R; Fernandes, M; Ranville, J (2018) SCIENCE OF THE TOTAL ENVIRONMENT 618: 1389-1399

Silver nanoparticles (Ag NPs) exponential production raises concern about their environmental impact. The effects of Ag NPs to aquatic plants remain scarcely studied, especially in extended exposures. This paper aims to evaluate Ag NPs effects in *Lemna minor* at individual and sub-individual levels, focusing on three variables: Ag form (NPs versus ions $Ag^+$), NPs surface coating (citrate vs polyvinylpyrrolidone - PVP) and exposure period (7 vs 14 days). Endpoints were assessed at individual level (specific growth rate, chlorosis incidence and number of fronds per colony) and sub-individual level (enzymatic activities of catalase (CAT), guaiacol peroxidase (GPx) and glutathione-S-transferase (GST)). Generally, plants exposed to all Ag forms underwent decays on growth rate and fronds per colony, and increases on chlorosis, GPX and GST, but no effects on CAT. The most sensitive endpoints were specific growth rate and GPx activity, showing significant effects down to 0.05 mg/L for Ag NPs and 3 µg/L for $Ag^+$, after 14 days. $Ag^+$ showed higher toxicity with a 14d-EC50 of 0.0037 mg Ag/L. Concerning surface coating, PVP-AgNPs were more deleterious on growth rate and fronds per colony, whereas citrate-Ag NPs affected more the chlorosis incidence and GPx and GST activities. The exposure period significantly affected chlorosis: 14 days triggered a chlorosis increase in $Ag^+$-exposed plants and a decrease in Ag NPs-exposed plants when compared to 7 days. Ag NPs induced an oxidative stress status in cells, thus ensuing upregulated enzymatic activity as a self-defense mechanism. Since Ag NPs dissolution might occur on a steady and continuous mode along time, and the average longevity of fronds, we propose longer exposures periods than the recommended by the OECD guideline. This approach would provide more relevant

and holistic evidences on the overall response of freshwater plants to Ag NPs in an ecological relevant scenario.

## Dissolved organic matter reduces CuO nanoparticle toxicity to duckweed in simulated natural systems

Rippner, DA; Green, PG; Young, TM; Parikh, SJ (2018) ENVIRONMENTAL POLLUTION 234: 692-698

With increasing demand for recycled wastewater for irrigation purposes, there is a need to evaluate the potential for manufactured nanomaterials in waste water to impact crop production and agroecosystems. Copper oxide nanoparticles (CuO NPs) have previously been shown to negatively impact the growth of duckweed (*Landoltia punctata*) a model aquatic plant consumed by water fowl and widely found in agricultural runoff ditches in temperate climates, However, prior studies involving CuO NP toxicity to duckweed have focused on systems without the presence of dissolved organic matter (DOM). In the current study, duckweed growth inhibition was shown to be a function of aqueous $Cu^{2+}$ concentration. Growth inhibition was greatest from aqueous $CuCl_2$ and, for particles, increased with decreasing CuO particle size. The dissolution of CuO NPs in 1/2 Hoagland's solution was measured to increase with decreasing particle size and in the presence of Suwannee river humic and fulvic acids (HA; FA). However, the current results suggest that HA, and to a lesser extent, FA, decrease the toxicity of both CuO NPs and free ionized Cu to duckweed, likely by inhibiting Cu availability through Cu-DOM complex formation. Such results are consistent with changes to Cu speciation as predicted by speciation modeling software and suggest that DOM changes Cu speciation and therefore toxicity in natural systems.

## Fate, toxicity and bioconcentration of cadmium on *Pseudokirchneriella subcapitata* and *Lemna minor* in mid-term single tests

Clement, B; Lamonica, D (2018) ECOTOXICOLOGY 27: 132-143

In the frame of a project which consists in modeling a laboratory microcosm under cadmium pressure, we initiated this study on the fate and effects of cadmium in the presence of either the microalga *Pseudokirchneriella subcapitata* or the duckweed *Lemna minor*, two organisms of the microcosm. For each organism, growth inhibition tests on a duration of 2-3 weeks were carried out with the objective of linking effects with total dissolved, ionic and internalized forms of cadmium. Numbers of organisms (algal cells or duckweed fronds) in 2-L beakers filled with synthetic nutritive medium containing EDTA were counted during the course of assays, while cadmium concentrations in the water and in the organisms were measured. Free cadmium fraction was calculated using PHREEQC, a computer program for chemical speciation. Results showed that cadmium toxicity to microalgae could be correlated to the free divalent fraction of this metal, limited by the presence of EDTA, and to its concentration in the organisms. Bioconcentration factors for our medium were suggested for *P. subcapitata* (111,000 on the basis of free cadmium concentration) and *L. minor* (17,812 on the basis of total dissolved concentration). No effect concentrations were roughly estimated around 400 µg/g for *Pseudokirchneriella subcapitata* and 200-300 µg/g for *Lemna minor*. This study is a first step towards a fate model based on chemical speciation for a better understanding of microcosm results.

## Toxic effects and mechanism of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) on *Lemna minor*

Qiu, NW; Wang, RJ; Sun, Y; Wang, XS; Jiang, DC; Meng, YT; Zhou, F (2018) CHEMOSPHERE 193: 711-719

To investigate the toxic effect and mechanism of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in aquatic plants, in vivo and in vitro exposure to BDE-47 were conducted. After 14-d exposure to 5-20 μg/L BDE-47, the growth of *Lemna minor* plants was significantly suppressed, and the chlorophyll and soluble protein contents in fronds markedly decreased. Accordingly, the photosynthetic efficiency (Fv/Fm, PI) decreased. When the thylakoid membranes isolated from healthy fronds was exposed to 5-20 mg/L BDE-47 directly in vitro for 1 h, the photosynthetic efficiency also decreased significantly. In both the in vitro (5-20 μg/L) and in vivo (5-20 mg/L) experiments, BDE-47 led to an increased plasma membrane permeability. Hence, we concluded that BDE-47 had a direct toxicity to photosynthetic membranes and plasma membranes. However, direct effects on the activities of peroxidase (POD), malate dehydrogenase (MDH) and nitroreductase (NR) were not observed by adding 5-20 mg/L BDE-47 into crude enzyme extracts. The malondialdehyde (MDA) and superoxide anion radical ($O^{-2}$) contents in the BDE-47 treated fronds were higher than those in the control fronds, suggesting that *L. minor* can not effectively relieve reactive oxygen species (ROS). The data above indicates that BDE-47 is toxic to *L. minor* through acting directly on biomembranes, which induces the production of ROS and thus causes remarkable oxidative damage to cells.

## Synthesis, crystal structure, molecular docking studies and bio-evaluation of some N-4-benzyl-substituted isatin-3-thiosemicarbazones as urease and glycation inhibitors

Pervez, H; Khan, N; Iqbal, J; Zaib, S; Yaqub, M; Tahir, MN; Naseer, MM (2018) HETEROCYCLIC COMMUNICATIONS 24: 51-58

Fifteen N-4-benzyl-substituted isatin-3-thiosem-icarbazones 5a-o were synthesized and evaluated for their urease and glycation inhibitory potential. *Lemna aequinocitalis* growth and *Artemia salina* assays were also done to determine their phytotoxic and toxic effects. All compounds are potent inhibitors of the urease enzyme, displaying inhibition [half maximal inhibitory concentration (IC50) = 1.08 ± 0.12, 11.23 ± 0.19 μM] superior to that of the reference inhibitor thiourea (IC50 = 22.3 ± 1.12 μM). Compounds 5c, 5d, 5h, 5j, k are potent antiglycating agents, showing glycation inhibitory activity better than that of the reference inhibitor rutin (IC50 values 209.87 ± 0.37, 231.70 ± 6.71 vs. 294.5 ± 1.5 μM). In the phytotoxicity assay, 11 thiosemicarbazones 5a-d, 5g, 5h, 5j-1, 5n, o are active, demonstrating 5-100% growth inhibition of *L. aequinocitalis* at the highest tested concentrations (1000 or 500 μg/mL). In the brine shrimp (*A. salina*) lethality bioassay, three derivatives 5b, 5j and 5o are active with median lethal dose (LD50) values of 3.63 x $10^{-5}$, 2.90 x $10^{-5}$ and 2.31 x $10^{-4}$ M, respectively.

## Assessment of the hazard of nine (doped) lanthanides-based ceramic oxides to four aquatic species

Blinova, I; Vija, H; Lukjanova, A; Muna, M; Syvertsen-Wiig, G; Kahru, A (2018) SCIENCE OF THE TOTAL ENVIRONMENT 612: 1171-1176

The risk of environmental pollution with rare earth oxides rises in line with increasing application of these compounds in different sectors. However, data on potential environmental hazard of

lanthanides is scarce and concerns mostly Ce and Gd. In this work, the aquatic toxicity of eight doped lanthanide-based ceramic oxides (Ce0.9Gd0.1O2, LaFeO3, Gd0.97CoO3, LaCoO3, (La0.5Sr0.5)(0.99)MnO3, Ce0.8Pr0.2O2, (La0.6Sr0.4)(0.95)CoO3, LaNiO4) and one non-doped oxide (CeO2) with primary size from 23 to 590 nm were evaluated in four short-term laboratory assays with freshwater crustaceans and duckweeds. Results showed no acute toxicity (EC50 > 100 mg/L) or very low acute toxicity for most studied oxides. Observed toxicity was probably due to bioavailable fraction of dopant metals (Ni and Co) but in the case of aquatic plants, decrease of nutrient availability (complexing of phosphorus by lanthanides) was also presumed. Studied oxides/metals accumulated in the aquatic plant tissue and in the gut of crustaceans and thus may be further transferred via the aquatic food chain. Accumulation of metals in the duckweed *Lemna minor* may be recommended as a cost-effective screening bioassay for assessment of potential hazard of poorly soluble oxides to aquatic ecosystems.

## Effect of graphene oxide on copper stress in *Lemna minor* L.: evaluating growth, biochemical responses, and nutrient uptake

Hu, CW; Liu, L; Li, XL; Xu, YD; Ge, ZG; Zhao, YJ (2018) JOURNAL OF HAZARDOUS MATERIALS 341: 168-176

The wide application and unique properties of graphene oxide (GO) make it to interact with other pollutants and subsequently alter their behavior and toxicity. We evaluated the influences of GO at different concentrations (1 and 5 mg/L) on copper (Cu) stress in duckweed (*Lemna minor* L.) GO below a concentration of 5 mg/L showed no adverse effects on *L. minor*. The addition of Cu above 10 jail represented a stress condition, which was evidenced by various parameters such as frond number, percent inhibition of growth rate (I-r), total chlorophyll content, dry weight, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). When L minor was simultaneously exposed to GO and Cu, especially at a GO concentration of 5 mg/L and a Cu level above 10 mu M, the increase of $I_r$ and decrease of chlorophyll content were inhibited, suggesting that the Cu stress was diminished in the presence of GO. The addition of Cu alone, ranging between 5 and 20 μM, increased Cu, B, Mn, Fe, Co, and Zn uptake, but decreased P uptake. Our results suggest that GO can lessen Cu stress in *L. minor* via Cu adsorption, thereby protecting the plants from the damaging effects of high Cu concentrations.

## Nanocapsulation of herbicide Haloxyfop-R-methyl in poly(methyl methacrylate): phytotoxicological effects of pure herbicide and its nanocapsulated form on duckweed as a model macrophyte

Torbati, S; Mahmoudian, M; Alimirzaei, N (2018) TURKISH JOURNAL OF CHEMISTRY 42: 132-145

In the present study the nanocapsulation of Haloxyfop-R-methyl in poly(methyl methacrylate) was successfully performed. Poly(methyl methacrylate)/Haloxyfop-R-methyl nanocapsules were synthesized using a miniemulsion method and their surface morphology was studied by scanning electron microscopy and transition electron microscopy. The chemical characterization of nanocapsules was done by FT-IR spectroscopy. The herbicide loading and encapsulation efficiency were also analyzed for the herbicide-loaded nanocapsules. In order to evaluate the toxic effects of nanocapsulated herbicide and pure herbicide on *Lemna minor* L., some physiological effects of these two compounds were investigated. The plant growth rate, photosynthetic pigment contents, and activities of antioxidant enzymes were evaluated as indices to assess the effects and toxicity of the encapsulated Haloxyfop-R-methyl and its nonencapsulated form on *Lemna minor*. The obtained results confirmed that the negative effects of encapsulated Haloxyfop-R-methyl on the physiological

parameters of *Lemna minor* were less than the effects of the pure herbicide. In the case of antioxidant enzymes activities, it was shown that all concentrations of the two examined groups led to the remarkable induction of superoxide dismutase activity as compared with the control sample. Possibly, the enzyme played an important role in the plant's resistance to the existence of the studied contaminants.

## The response of duckweed (*Lemna minor* L.) roots to Cd and its chemical forms

Xue, Y; Wang, JQ; Huang, J; Li, FY; Wang, M (2018) JOURNAL OF CHEMISTRY Article Number: 7274020; DOI: 10.1155/2018/7274020

The response of duckweed (*Lemna minor* L.) roots to Cd and its chemical forms was investigated. The relative root growth rate and concentrations of Cd and its different chemical forms in the root, that is, ethanol-extractable ($F_E$-Cd), HCl-extractable ($F_{HCl}$-Cd), and residual fractions ($F_r$-Cd), were quantified. Weibull model was used to unravel the regression between the relative root elongation (RRL) with chemical forms of Cd. Parameters assessed catalase (CAT), peroxidases (POD), and superoxide dismutase (SOD), as well as malondialdehyde (MDA) and total antioxidant capacity (A-TOC). Our results show that both the relative root growth rate and relative frond number were affected by Cd concentrations. The chemical forms of Cd were influenced by Cd content in the medium. Relative root elongation (RRL) showed a significant correlation with chemical forms of Cd. Additionally, POD and SOD increased at lower Cd concentrations followed by a decrease at higher Cd concentrations (at more than 5 μM Cd). Moreover, MDA and A-TOC increased and CAT decreased with increasing Cd exposure. Furthermore, CAT showed a significant correlation with $F_{HCl}$-Cd. Taken together, it can be concluded that the chemical forms of Cd are statistically significant predictors of Cd toxicity to duckweed and to the other similar aquatic plants.

## Chronic Toxicity of Aluminum, at a pH of 6, to Freshwater Organisms: Empirical Data for the Development of International Regulatory Standards/Criteria

Cardwell, AS; Adams, WJ; Gensemer, RW; Nordheim, E; Santore, RC; Ryan, AC; Stubblefield, WA (2018) ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 37: 36-48

The chemistry, bioavailability, and toxicity of aluminum (Al) in the aquatic environment are complex and affected by a wide range of water quality characteristics (including pH, hardness, and dissolved organic carbon). Data gaps in Al ecotoxicology exist for pH ranges representative of natural surface waters (pH 6-8). To address these gaps, a series of chronic toxicity tests were performed at pH 6 with 8 freshwater species, including 2 fish (*Pimephales promelas* and *Danio rerio*), an oligochaete (*Aeolosoma* sp.), a rotifer (*Brachionus calyciflorus*), a snail (*Lymnaea stagnalis*), an amphipod (*Hyalella azteca*), a midge (*Chironomus riparius*), and an aquatic plant (*Lemna minor*). The 10% effect concentrations (EC10s) ranged from 98 mg total Al/L for D. rerio to 2175 mg total Al/L for *L. minor*. From these data and additional published data, species-sensitivity distributions (SSDs) were developed to derive concentrations protective of 95% of tested species (i.e., 50% lower confidence limit of a 5th percentile hazard concentration [HC5-50]). A generic HC5-50 (not adjusted for bioavailability) of 74.4mg total Al/L was estimated using the SSD. An Al-specific biotic ligand model (BLM) was used to develop SSDs normalized for bioavailability based on site-specific water quality characteristics. Normalized HC5-50s ranged from 93.7 to 534mg total Al/L for waters representing a range of European ecoregions, whereas a chronic HC5 calculated using US Environmental Protection Agency aquatic life criteria methods (i.e., a continuous criterion concentration [CCC]) was

125mg total Al/L when normalized to Lake Superior water in the United States. The HC5-50 and CCC values for site-specific waters other than those in the present study can be obtained using the Al BLM.

## Is the Tier-1 Effect Assessment for Herbicides Protective for Aquatic Algae and Vascular Plant Communities?

van Wijngaarden, RPA; Arts, GHP (2018) ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 37: 175-183

In the aquatic tier-1 effect assessment for plant protection products with an herbicidal mode of action in Europe, it is usually algae and/or vascular plants that determine the environmental risks. This tier includes tests with at least 2 algae and 1 macrophyte (*Lemna*). Although such tests are considered to be of a chronic nature (based on the duration of the test in relation to the life cycle of the organism), the measurement endpoints derived from the laboratory tests with plants (including algae) and used in the first-tier effect assessment for herbicides are acute effect concentrations affecting 50% of the test organisms (EC50 values) and not no-observed-effect concentrations (NOECs) or effect concentrations affecting 10% of the test organisms (EC10) values. Other European legislative frameworks (e.g., the Water Framework Directive) use EC10 values. The present study contributes to a validation of the tiered herbicide risk assessment approach by comparing the standard first-tier effect assessment with results of microcosm and mesocosm studies. We evaluated EC50 and EC10 values for standard test algae and macrophytes based on either the growth rate endpoint ($E_rC50$) or the lowest available endpoint for growth rate or biomass/yield ($E_r/E_yC50$). These values were compared with the regulatory acceptable concentrations for the threshold option as derived from microcosm and mesocosm studies. For these studies, protection is maintained if growth rate is taken as the regulatory endpoint instead of the lowest value of either growth rate or biomass/yield in conjunction with the standard assessment factor of 10. Based on a limited data set of 14 herbicides, we did not identify a need to change the current practice.

## Comparative investigation of toxicity and bioaccumulation of Cd-based quantum dots and Cd salt in freshwater plant *Lemna minor* L.

Modlitbova, P; Novotny, K; Porizka, P; Klus, J; Lubal, P; Zlamalova-Gargosova, H; Kaiser, J (2018) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 147: 334-341

The purpose of this study was to determine the toxicity of two different sources of cadmium, i.e. $CdCl_2$ and Cd-based Quantum Dots (QDs), for freshwater model plant *Lemna minor* L. Cadmium telluride QDs were capped with two coating ligands: glutathione (GSH) or 3-mercaptopropionic acid (MPA). Growth rate inhibition and final biomass inhibition of *L. minor* after 168-h exposure were monitored as toxicity endpoints. Dose-response curves for Cd toxicity and EC50(168h) values were statistically evaluated for all sources of Cd to uncover possible differences among the toxicities of tested compounds. Total Cd content and its bioaccumulation factors (BAFs) in *L. minor* after the exposure period were also determined to distinguish Cd bioaccumulation patterns with respect to different test compounds. Laser-Induced Breakdown Spectroscopy (LIBS) with lateral resolution of 200 μm was employed in order to obtain two-dimensional maps of Cd spatial distribution in *L. minor* fronds. Our results show that GSH- and MPA-capped Cd-based QDs have similar toxicity for *L. minor*, but are significantly less toxic than $CdCl_2$. However, both sources of Cd lead to similar patterns of Cd bioaccumulation and distribution in *L. minor* fronds. Our results are in line with previous reports that the main mediators of Cd toxicity and bioaccumulation in aquatic plants are $Cd^{2+}$ ions dissolved from Cd-based QDs.

## Alleviation of cadmium toxicity in *Lemna minor* by exogenous salicylic acid

Lu, QQ; Zhang, TT; Zhang, W; Su, CL; Yang, YR; Hu, D; Xu, QS (2018) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 147: 500-508

Cadmium (Cd) is a significant environmental pollutant in the aquatic environment. Salicylic acid (SA) is a ubiquitous phenolic compound. The goal of this study was to assess the morphological, physiological and biochemical changes in duckweed (*L. minor*) upon exposure to 10 μM $CdCl_2$, 10 μM $CdCl_2$ plus 50 μM SA, or 50 μM SA for 7 days. Reversing the effects of Cd, SA decreased Cd accumulation in plants, improved accumulation of minerals (Ca, Mg, Fe, B, Mo) absorption, increased endogenous SA concentration, and phenylalanine ammonialyase (PAL) activity. Chlorosis-associated symptoms, the reduction in chlorophyll content, and the overproduction of reactive oxygen species induced by Cd exposure were largely reversed by SA. SA significantly decreased the toxic effects of Cd on the activities of the superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase in the fronds of *L. minor*. Furthermore, SA reversed the detrimental effects of Cd on total ascorbate, glutathione, the ascorbic acid/oxidized dehydroascorbate and glutathione/glutathione disulphide ratios, lipid peroxidation, malondialdehyde concentration, lipoxygenase activity, and the accumulation of proline. SA induced the up-regulation of heat shock proteins (Hsp70) and attenuated the adverse effects of Cd on cell viability. These results suggest that SA confers tolerance to Cd stress in *L. minor* through different mechanisms.

## Recovery of *Lemna minor* after exposure to sulfadimethoxine irradiated and non-irradiated in a solar simulator

Drobniewska, A; Wojcik, D; Kapan, M; Adomas, B; Piotrowicz-Cieslak, A; Nalecz-Jawecki, G (2017) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 27642-27652

Sulfonamides are the second most widely used group of veterinary antibiotics which are often detected in the environment. They are eliminated from freshwaters mainly through photochemical degradation. The toxicity of sulfadimethoxine (SDM) was evaluated with the use of *Lemna minor* before and after 1- and 4-h irradiation in a SunTest CPS+ solar simulator. Eight endpoints consisting of: number and total area of fronds, fresh weight, chlorophylls a and b, carotenoids, activity of catalase and guaiacol peroxidase, and protein content were determined. The total frond area and chlorophyll b content were the most sensitive endpoints with EC50 of 478 and 554 μg $L^{-1}$, respectively. The activity of guaiacol peroxidase and catalase increased at SDM concentrations higher than 125 and 500 μg $L^{-1}$, respectively. The SDM photodegradation rate for first order kinetics and the half-life were 0.259 $h^{-1}$ and 2.67 h, respectively. The results show that the toxicity of irradiated solutions was caused by SDM only, and the photoproducts appeared to be either non-toxic or much less toxic to *L. minor* than the parent compound. To study the recovery potential of *L. minor*, after 7 days exposure in SDM solutions, the plants were transferred to fresh medium and incubated for the next 7 days. *L. minor* has the ability to regenerate, but a 7-day recovery phase is not sufficient for it to return to an optimal physiological state.

## Cytotoxic, Phytotoxic and Insecticidal Activities of *Chrysophthalmum montanum* (DC.) Boiss

Ayaz, F; Kucukboyaci, N; Duman, H; Sener, B; Choudhary, MI (2017) TURKISH JOURNAL OF PHARMACEUTICAL SCIENCES 14: 290-293

The crude methanol (80%) extract of the aerial parts of *C. montanum* was fractionated to obtain n-hexane, chloroform, n-butanol, and remaining water fractions. The crude extract and subsequent solvent fractions of the plant were evaluated for their biological activities using screening bioassays such as cytotoxicity on brine shrimp lethality, phytotoxicity against *Lemna minor* L., and insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum*. The cytotoxicity assay revealed that the crude extract, n-hexane, and chloroform fractions of the plant had positive lethality with LD50 values of 71.51, 126.62, and 75.95 µg/mL, respectively. The extract and its fractions, except for the remaining water fraction, showed phytotoxic activity, which was expressed as percentage growth regulation in a concentration-dependent manner. n-hexane and chloroform fractions in particular had 100% growth inhibition (GI) at 1000 µg/mL, followed by the n-butanol fraction (62.6% GI) and crude extract (40.0% GI) of the plant at the same concentration. Otherwise, all samples had no insecticidal activity against *R. dominica* and *T. castaneum*. This study demonstrates that *C. montanum* contains bioactive compounds related to potential biological activities such as cytotoxic and phytotoxic.

## Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery-Biological effects and stability under test conditions

Bialk-Bielinska, A; Mulkiewicz, E; Stokowski, M; Stolte, S; Stepnowski, P (2017) CHEMOSPHERE 189: 689-698

Available ecotoxicological data for anti-cancer drugs and their metabolites are incomplete, and only some studies have been accompanied by chemical analysis. Therefore, the main aim of this study was to evaluate the acute toxicity of the six most commonly used cytostatics, namely cyclophosphamide (CF), ifosfamide (IF), 5-fluorouracil (5-FU), imatinib (IMT), tamoxifen (TAM) and methotrexate (MET) and its metabolite - 7-hydroxymethotrexate (7-OH-MET), towards selected aquatic organisms, namely bacteria *Vibrio fischeri*, algae *Raphidocelis subcapitata*, crustaceans *Daphnia magna* and duckweed *Lemna minor*. All ecotoxicological tests were accompanied by chemical analysis to determine the differences between nominal and actual concentrations of investigated compounds and their stability under test conditions. For unstable compounds, tests were performed in static and semi-static conditions. It was observed that *L. minor* was the most sensitive organism. The compounds that were most toxic to aquatic organisms were 5-FU (highly toxic to algae, EC50 = 0.075 mg $L^{-1}$), MET and TAM (very toxic to highly toxic to duckweed depending on the test conditions; EC50MET 0.08-0.16 mg $L^{-1}$, EC50TAM 0.18-0.23 mg $L^{-1}$). It is suspected that MET and 5-FU mainly affected algae and plants most probably because the exposure time was long enough for them to cause a specific effect (they inhibit DNA replication and act predominantly on actively dividing cells). Furthermore, the obtained results also suggest that the toxicity of the metabolites/potentially produced degradation products of MET towards duckweed is lower than that of the parent form, whereas the toxicity of TAM degradation products is in the same range as that of TAM.

# Taxonomy

## Fingerprinting by amplified fragment length polymorphism (AFLP) and barcoding by three plastidic markers in the genus *Wolffiella* Hegelm

Bog, M; Landrock, MF; Drefahl, D; Sree, KS; Appenroth, KJ (2018) PLANT SYSTEMATICS AND EVOLUTION 304: 373-386

Amplified fragment length polymorphism (AFLP) fingerprinting and three different plastidic DNA regions (rpl16, rps16, atpF-atpH) were used to investigate species identity in the genus *Wolffiella*. For this purpose, clones (67 in total) belonging to all ten species were selected. Almost all the species were represented by more than one clone. The fingerprinting technique, AFLP, clearly distinguished the species, *W. caudata, W. gladiata, W. neotropica, W. rotunda*, and *W. welwitschii*. Apart from confirming the molecular identity of these five species, the plastidic markers could delineate two additional species, *W. hyalina* and *W. denticulata*, although the conclusion concerning the latter is restricted by the availability of only one clone. The efficiency of the plastid-derived markers in identifying the number of species-specific clusters followed the sequence rps16 > rpl16 > atpF-atpH. The species *W. lingulata, W. oblonga*, and *W. repanda* could not be identified by any of the molecular methods presented here, but could be strictly defined on a morphological basis. In several clones, high amounts of genetic admixtures between different species were detected. Further, simulation studies demonstrated that these clones are genetic hybrids. This might be one of the obstacles in molecular identification of species in the genus *Wolffiella*.

## Taxonomic survey of the Araceae Juss. in the coastal region of Piaui state, northeast Brazil, including the Rio Parnaiba Delta

Freitas, RN; Silva, MFS; Paiva, JS; Mayo, SJ; de Andrade, IM (2017) IHERINGIA SERIE BOTANICA 72: 341-350

This is the first taxonomic study of the family Araceae in the state of Piaui, Northeast Brazil. The survey was conducted in four coastal municipalities (Ilha Grande, Luiz Correia, Cajueiro da Praia and Parnaiba) of the state. Eight species were recorded in six genera: *Lemna aequinoctialis* Welw., *L. valdiviana* Phil., *Montrichardia linifera* (Arruda) Schott, *Pistia stratiotes* L., *Wolffiella lingulata* (Hegelm.) Hegelm., *W. oblonga* (Phil.) Hegelm., *Taccarum ulei* Engl. & K. Krause and *Spathicarpa gardneri* Schott. Of these, two are geophytes (*T. ulei*, *S. gardneri*) and the others are aquatic macrophytes. Descriptions, illustrations, phenology and habitat information are given for each species as well as a key to their identification.

## Phylogenic study of Lemnoideae (duckweeds) through complete chloroplast genomes for eight accessions

Ding, YQ; Fang, Y; Guo, L; Li, ZD; He, KZ; Zhao, Y; Zhao, H (2017) PEERJ 5: Article Number: e4186; DOI: 10.7717/peerj.4186

Phylogenetic relationship within different genera of Lemnoideae, a kind of kind of small aquatic monocotyledonous plants, was not well resolved, using either morphological characters or traditional markers. Given that rich genetic information in chloroplast genome makes them particularly useful for phylogenetic studies, we used chloroplast genomes to clarify the phylogeny

within Lemnoideae. DNAs were sequenced with next-generation sequencing. The duckweeds chloroplast genomes were indirectly filtered from the total DNA data, or directly obtained from chloroplast DNA data. To test the reliability of assembling the chloroplast genome based on the filtration of the total DNA, two methods were used to assemble the chloroplast genome of *Landoltia punctata* strain ZH0202. A phylogenetic tree was built on the basis of the whole chloroplast genome sequences using MrBayes v.3.2.6 and PhyML 3.0.

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    - 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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