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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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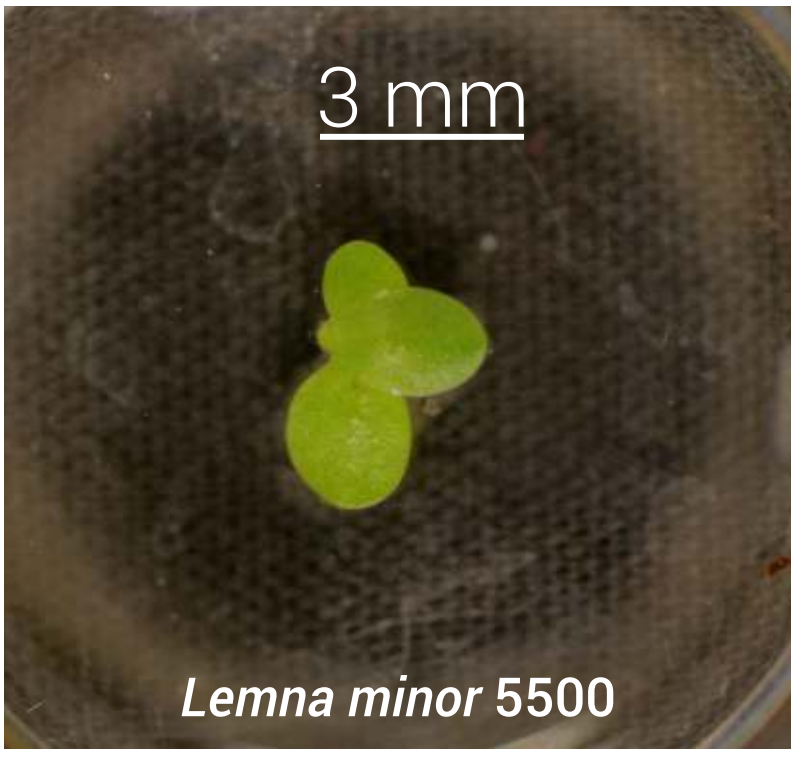

Representatives for four species of duckweed are shown. The genome sequence for 3 of these strains have been either published (*L. minor* 5500) or are currently being completed and publication submitted (*L. gibba* G3 7741 and *S. polyrhiza* 9509). In addition, these species comprise some of the most commonly used model duckweeds for various research that have been published. For example, *L. minor* has 1,567 hits on the WebOfSciences database and is often used for water toxicity tests using the ISO20079 protocol. All photos are taken under identical magnification with the scale bar (3 mm) shown. Photographs taken by Dr. Eric Lam at the RDSC (Rutgers University).

In this issue

International Steering Committee on Duckweed Research and Applications Members

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

Letter from the editor

Dear Friends of Duckweed,

Until recently, ISCDRA was represented by a drawing of *Wolffiella gladiata* made by Dr. K. Sowjanya Sree, India. Some time back, the ISCDRA decided to have a logo especially designed for it. Now the time has arrived when we can use it for the first time in this issue of our "Duckweed Forum". We hope all of you will enjoy it. The new logo is a gift from the Argentinean artist, Ms. Romina C. Romano. We are very grateful to her. Along with the new logo, this issue also portrays about the artist and her work. In the same issue additionally, Mr. Eduardo Mercovich reports about MamaGrande in Argentina and how this company is building one of the 1st biorefineries created to produce biopolymers from duckweed.

At the end of our 3rd duckweed meeting in Kyoto, Prof. Dr. Eric Lam stressed in the farewell address on the necessity to develop scientific standards in the field of duckweed research in order to compete with other research fields. We report in the Discussion Corner about a very recent publication, which tries to develop this idea by describing the taxonomic situation in the plant family of duckweeds. The publication lists the out-dated names of species otherwise called synonyms together with the currently valid nomenclature.

As Student Spotlight we introduce Dr. Manuela Bog from the University of Regensburg, Germany and we can read how much she likes duckweed. This is followed by new duckweed experiments for students and useful methods in duckweed research.

Lastly, as suggested by ISCDRA member Prof. Eric Lam, we have started with this issue of DF a new cover design that will present color photos for 4 different species of duckweed. The aim is to showcase the variety of morphologies that are observed in the duckweed family - from the largest Spirodela to the smallest Wolffia. In a little over 9 issues of the DF, we should be able to cover and showcase all 37 known species of duckweed and provide an accessible visual reference for everyone interested in duckweed.

As always you can update with the abstracts of the newest duckweed publications in the fields of Biotechnology, Ecology, Feed and Food, Physiology, Phytomedicine, Phytoremediation and (the largest part) Phytotoxicity. See also the two selected highlight papers from the last few months.

Best wishes to all of you.

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

Discussion corner

Dear Reader of the Newsletter.

At the end of the 3rd International Conference on Duckweed Research and Applications, in Kyoto Japan 2015, Professor Eric Lam delivered a final address. He not only thanked the organizers from Japan for their work in preparing the conference, he also gave some ideas about what should be done in the near future. One point he stressed is the requirement for scientific standards in our research as a precondition to attract researchers from other fields. Just to give an example, I often review manuscripts for journals in which as the source of the investigated duckweed species is given as a nearby pond. Try a similar description when you work with *Arabidopsis thaliana* or any other plant and each editor will reject the manuscript without reviewing it. A minimum requirement is the registration of the clone at Rutgers Duckweed Stock Cooperative at the New Jersey State University. We installed a comfortable way for doing it and we ask all authors and reviewers taking care of this point.

Another very important point is the use of correct and valid names for duckweed species. When you read the literature, you will find almost humorous terms like *Lemna polyrhiza* or *Lemna arrhiza*. Species of the genus *Lemna* are defined by having one single root per frond. “polyrhiza” and “arrhiza” are a Greek terms meaning “many roots” and “without roots”, respectively. Thus, the mentioned terms are not only not valid, they are simply a contradiction in themselves. You might find many other examples of invalid names for species in the literature. This spoils our image as duckweed community.

Therefore, we have published a manuscript with the title:

Taxonomy of duckweeds (Lemnaceae), potential new crop plants (Review)

K. Sowjanya Sree, Manuela Bog, Klaus-Juergen Appenroth

Emirates Journal of Food and Agriculture. 2016, 28(5): 291-302.

doi: 10.9755/ejfa.2016-01-038, http://www.ejfa.me/

Abstract

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

This paper gives all correct names of duckweeds and a list of synonyms (i.e. not valid names) with the correct references. The paper has open access and will be available within the next few days. Please, check the name of your own species and cite this paper to support us.

Klaus-J. Appenroth, University of Jena, Germany

MamaGrande's 1st biorefinery

Some context

MamaGrande[1] is an Argentinean social biotech company founded in 2011. The company uses duckweed as the technology platform to couple wastewater remediation with renewable and biodegradable biopolymer generation (Polylactic Acid, or PLA, one of the most widely known and adaptable renewable and biodegradable biopolymers on our planet today[2]).

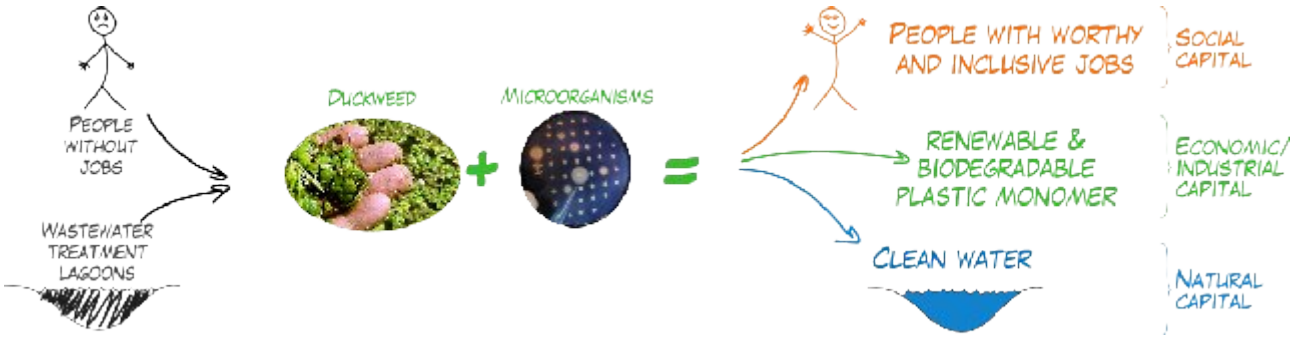

Illustration 1: Simplified diagram of MamaGrande's process

The interesting point about this apparently strange marriage is that today PLA is done with corn or other source of food as raw material, competing with food production not only for water, but for soil surface too. MamaGrande's operations, on the contrary, arise from biomass that is produced **while cleaning water** and **without competition with food production whatsoever**.

Following our plan described previously[3], we've been scaling up from our pilot in a small town with 11,000 inhabitants to a sizable province with ~2,000,000 people and are building our 1st biorefinery.

1 For more information on MamaGrande (including a TEDx talk with english subtitles) see: http://MamaGrande.org
2 Polylactic Acid, or PLA: https://en.wikipedia.org/wiki/Polylactic_acid and http://onlinelibrary.wiley.com/doi/10.1111/j.1541-4337.2010.00126.x/full
3 See earlier articles of the Duckweed Forum in http://lemnapedia.org/wiki/ISCDRA

Illustration 2: Small (~1ha) secondary wastewater lagoon at El Carril in Salta, Argentina.

In almost any - but particularly the biotechnology - industry, scale-up is often a complex and challenging process, done usually by 10-fold increments from the lab bench, to a pilot, then to an industrially-relevant small to big sizes (see table: Scale up phases and characteristics). Each step has its own challenges and results, being all of them important to reach our social, natural and economic impact targets.

View-scale	Lab	Pilot	Industrial
Scale	ml	10-10^2 liters	> 10^3 liters (m^3)
Results	knowledge	working example	Products
Required flexibility	high	medium	low
Predictability	low	medium	high

Table 1: Scale up phases and characteristics

In Salta (one of the NW Argentinean provinces), we already started to modify some wastewater lagoons as part of this scale-up process. This field work advances as expected, gaining experience and knowledge in the development of this new cropping system that produces clean water instead of using it, while producing protein or carbohydrates for other uses by using energy from the sun. However, since these industrial and construction processes take a long time, we started almost a year ago to define and design our 1st biorefinery.

The Biorefinery

Being a pilot between the lab and full industrial scale, this biorefinery has some interesting aspects.

It has 2 fermenters: 200 and 1,000 liters in capacity. This allows us to work on the whole range from the lab to a full industrial scale.

Illustration 3: The 200 and 1000 lt fermenters, in revision before the final tests.

Both fermenters have a large array of sensors, with logging and actuator/control hardware and software. This combination provides us with real-time data and changes through the Internet. This data-intensive setup will allow us not only to traverse the physical scale from ml to thousands of liters, but also the different needs starting from learning, to scaling up such a vast and complex process: from wastewater to renewable & biodegradable polymers (with monomeric lactic acid as our 1st bioproduct).

Illustration 4: Testing some logging and control software.

Physically, the plant has a few different areas:

- an inoculation (pharma grade) lab,
- the biomass pretreatment area,
- the fermenters, and
- purification.
- Some auxiliary services like, boiler and chiller, air compressor, reverse osmosis filters, effluent neutralization and treatment, etc..

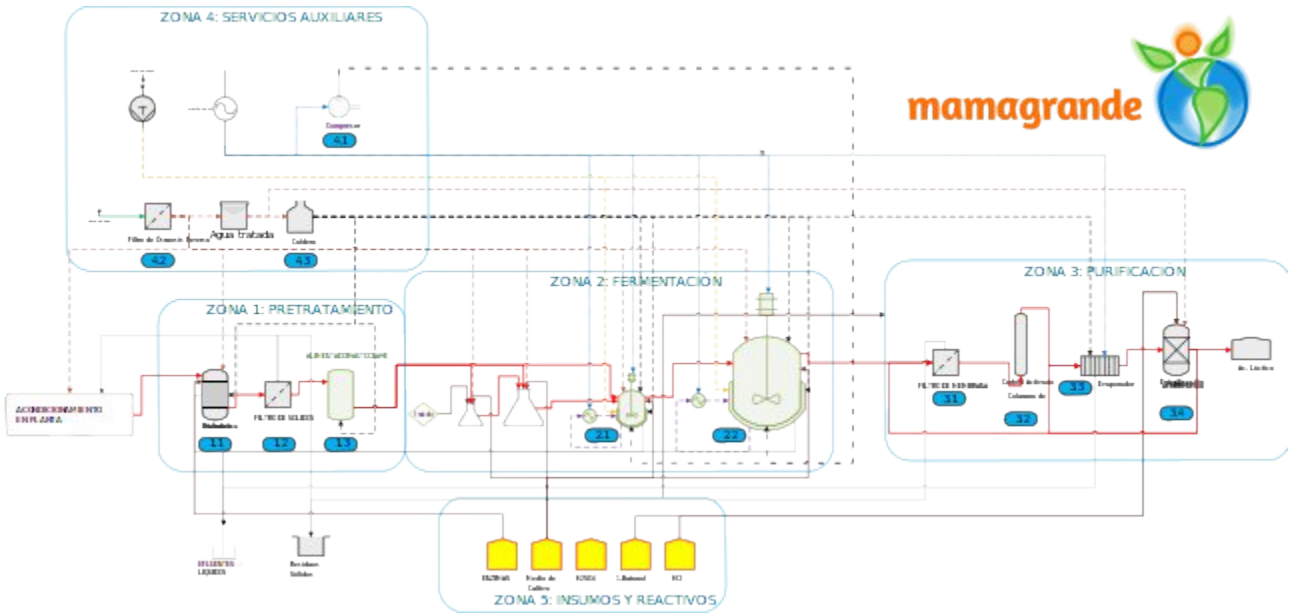

Illustration 5: MamaGrande's biorefinery schematics

Next steps

The inoculation lab, auxiliary services and fermenters were already built and tested on its development site in Junín (province of Buenos Aires, Argentina) with local workers and expertise.

Illustration 6: One of the many test runs.

As we write this article (April 2016), a specialized crew is starting its transfer to our R&D facilities in Rosario.

Following our open innovation model (i.e. no patents but publications), this pilot plant will be available to the wider scientific and start-up communities for other similar developments for a reasonable operating fee. We have been quite fortunate that even before the operations started we already have some requests from local biotech firms to use some of our facilities.

Illustration 7: Small panoramic view of the fermentors with their supporting equipment.

This biorefinery will remain in Rosario to aid in completing our own scale-up and before the end of 2017 will be moved to Salta, where we hope will give many years of faithful service to the whole biosphere in that region of Argentina.

If anyone feels piqued with these issues, may it the biorremediation phase or the industrial process phase, the ILA[4] is organizing a field trip to our operations in Salta (Argentina) by the end of 2016. Also of interest in Salta are her renowned empanadas, asados and some unforgettable wines from the famous Andes vineyards. Interested parties should contact Tamra Fakhoorian <tamraf9@gmail.com> for arrangements.

4 International Lemna Association, http://www.internationallemnaassociation.org

ISCDRA has a new logo

Romina Romano; rr@rominaromano.com.ar

(This is the original text in Spanish, it is followed by the English translation.)

Soy una profesional del campo de la imagen y la comunicación. Ideo, planifico y desarrollo todo tipo de emprendimientos gráficos y visuales en sus aspectos tanto publicitarios como tecnológicos.

Baso mi trabajo en un profundo análisis del mensaje a comunicar, atendiendo a sus necesidades concretas de expresividad y funcionalidad, comprometiendo todo a un diseño creativo, coherente e innovador para lograr soluciones emocionalmente evocativas.

Parte de mi filosofía de trabajo es la colaboración con proyectos de bien común, particularmente los que estén relacionados con el cuidado de nuestro ambiente u orientados a mejorar la calidad de vida de las personas.

Mi decisión de participar en el proyecto de creación de la marca de ISCDRA viene ligada a mi relación personal y laboral con Eduardo Mercovich, al cual conozco desde hace muchos años y junto al que he vivenciado hermosas experiencias de trabajo grupal.

Espero que mi aporte haya sido al menos un granito de arena más, para poder estar cada dia mas cerca de vivir en un mundo mejor.

I am a visual communications professional. I formulate, plan and develop both the marketing and technological aspects of many graphics projects.

I base my work on a deep analysis of the message that we must communicate, attending to its real expressive and functional needs, and committing everything to a creative, coherent and innovative design to arrive at solutions that are emotionally evocative.

Part of my life/work philosophy is the collaboration with projects for the common good, specially if they are related to the environment or dedicated to raising people's quality of life.

My participation in the project to create the ISCDRA brand is closely related with my personal and professional relationship with Eduardo Mercovich, whom I know since many years ago and with whom I enjoyed beautiful team-work experiences.

I hope that my work has been at least a small grain of sand in our common path to a better world.

Student Spotlight - Manuela Bog

Manuela Bog, University of Regensburg, Institute of Plant Sciences, Regensburg, Germany; manuela.bog@biologie.uni-regensburg.de

Already at a young age, I was interested in natural sciences and environment. A professional practical training during my time at school led me to the natural history museum of my home town. It was a lot of fun and I spent many voluntary hours working there during my holidays. As biology was also one of my favorite subjects at school it was obvious to enroll as a student for a diploma program in biology at the University of Jena (Germany). At the beginning of my studies I did not focus on plant sciences and I never thought to become a botanist. This attitude changed rapidly and I got highly interested in how plants cope with their biotic and abiotic environment and how plants interact with each other or even with the other kingdom's members.

During that time I also met Dr. Klaus Appenroth. I was very interested in his lectures and research. Finally, a practical course on heavy metal stress in duckweed was the crucial factor to write my thesis at the Appenroth lab in 2009. The topic was about molecular characterization and barcoding in duckweed using DNA sequencing and AFLP-fingerprinting, to improve species determination. We found that most species can be determined by sequencing certain chloroplast regions, which is a hard job on a morphological basis.

For my PhD studies I changed to the lab of Prof. Dr. C. Oberprieler at the University of Regensburg (Germany). Here, I was working on the consequences of hybridization in European ragwort species on their genetics, phytochemistry and susceptibility to herbivores. Although it had nothing to do with duckweed, I could broaden my previous knowledge and additionally, I learned many new aspects about systematics and evolution, especially about hybridization and polyploidization as important evolutionary processes. By the way, I kept in touch with my former supervisor and today's colleague Klaus Appenroth, which led to a further contribution to the duckweed research from my side.

In September 2015, I finished my PhD and I decided to continue with research on duckweed as they are remarkable plants with potential economic benefits and they bring along some advantages in the lab like easy and handy cultivation or fast clonal growth. I am looking forward using a broad spectrum of molecular methods to investigate evolutionary and developmental processes as well as stress phenomena that I partially applied already recently.

Experiments for students 4: Selective effects of herbicides on Rosopsida and Liliopsida

Goal: The effects of an auxin-like herbicide will be investigated within two parts of experiments: (Part A) Effect on a mixed culture of *Rosopsida/ Liliopsida*, (Part B) Effect on a culture of duckweed (*Liliopsida*).

Materials

Part A: For each group of students 2 pots with some seedlings of cereals (e.g. wheat) mixed in the same pot with seedlings of mustard (*Sinapis alba*) The seedlings should be approximately 14 days old.

Part B. Autotrophic culture of duckweed. Recommended: *Wolffia arrhiza* or *Wolffia globosa*. Also other species can be used (see below).

Chemicals: No special chemicals required. Nutrient medium is given below.

Equipment:

1. Two sprayers as used for hair styling (for herbicide solution and control)

2. Inoculation needle for duckweed

3. Graduaded cylinder, 50 ml

4. Burner (gas or spiritus)

5. For each student group 2 100 ml-Erlenmeyer flasks, if possible autoclaved

Ready-to use solutions:

1. 0.05% solution of 2,4-Dichloro phenoxyacetic acid (= 2,4-D) in water for spraying on the plants in part A.

2. 1 x 100 ml-Erlenmeyer flask per student group with 50 ml of 1 µM 2,4-D in nutrient medium.

3. 1 x 100 ml-Erlenmeyer flask per student group filled with 50 ml nutrient medium without herbicide.

Accomplishment

Part A) Flower pots with soil or vermiculite containing mixed cultures: mixed culture is sprayed with (i) 2,4-D solution, (ii) water (control).The water control should be used by several student groups together. The experiment can be extended by spraying a third pot of mixed culture with 10 µM aqueous solution of auxin.

Processing time: approximately 10 min

Part B) A defined number of fronds (e.g. 5) were transferred with an inoculating needle into 100 ml-Erlenmeyer flask containing 50 ml nutrient medium. This experiment does not require a sterile bench as no sugar is used in the nutrient medium.

In a similar way, the same number of fronds are inoculated in nutrient medium containing the herbicide (1 µM 2,4-D). Take care not to contaminate the control flasks with the herbicide.

Processing time: approximately 15 min

Plants were cultivated under day light conditions (if available use green house). After 7 days of cultivation the fronds are evaluated and the number of them counted. Remember, that number of colonies is not the same as number of fronds. Also measuring fresh and/ or dry weight is possible.

Evaluation

In both parts, A and B, the appearance of the plants should be described.

In part A) the number of survived and died plants (*Liliopsida* and *Rosopsida*, according to older nomenclature = monocots and dicots) should be given. What is the reason of the difference between both species?

In part B) the number of plants at the start and after the cultivations should be given and used to calculate the relative growth rates (cf. issue of the (Duckweed Forum 3, 180-186 (2015)).

Compare the results of part B with the responses of the two species in part A, and discuss the reason!

Selective herbicides like 2,4-D affect differently monocots and dicots!

Remark about the species selection: Basically, any of the duckweed species can be used. Those from the genera *Wolffia* and *Wolffiella* do not have roots. It might be that roots take up the herbicide, regardless whether they belong to *Liliosida* or *Rosopsida*. This would spoil the interpretation.

Remark about the nutrient medium: Any duckweed medium can be used in this experiment. We are using the so-called N-medium containing 8 mM KNO3, 0.06 mM KH2PO4, 1 mM MgSO4, 1 mM Ca(NO3)2, 5 µM H3BO3, 0.4 µM Na2MoO4, 13 µM MnCl2, 25 µM Fe(III)NaEDTA (Appenroth et al. 1996). For this reason, see other protocols in our newsletter (DuckweedForum 3, 180-186 (2015)).

Explanation for teachers

Herbicides like 2,4-D act on dicots (*Rosopsida*) but not on monocots (*Liliopsida*). There are different mechanisms (expositions of leafs toward herbicide applications; protection of meristematic tissues in monocots, leaf area, uptake, translocation) for this effect. The experiment shows that prevention of uptake in monocots through the cuticle layer is an essential mechanism. Although duckweed belong to monocots, they were killed by the herbicide since as aquatic plants they hardly have any cuticle layer.

We suggest rootless duckweeds because the uptake by roots might be unspecific (this effect has not been investigated in duckweeds). However, neglecting this possible disturbance, also other species like *Spirodela polyrhiza* can be used.

Useful Methods 5: DNA isolation

DNA Extraction with CTAB buffer for amplified fragment length polymorphism (AFLP) and sequencing of PCR-amplified markers

Klaus-J. Appenroth, Institute of Plant Physiology, University of Jena, Jena, Germany

Manuela Bog, University of Regensburg, Institute of Plant Sciences, Regensburg, Germany

Reference: Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11-15

Isolation of DNA for AFLP or analyzing plastidic markers required standardization but no complicated technique. We used expense column techniques (Qiagen) but the present method is cheaper and fulfills all requirements. Starting point in each case is frozen fresh material.

Protocol

- grind 100 mg tissue in a mortar in liquid nitrogen
- pour leaf powder into centrifuge tube
- after liquid nitrogen has evaporated, add 3.5 ml CTAB extraction buffer, shake gently and collect tubes on ice
- add beta-Mercaptoethanol (4 µl/tube)
- 30 min at 60°C in water bath
- optional: centrifugation at ca. 6000 g for 10 min, keep supernatant
- add 3 ml Chloroform:Isoamyl-alcohol (24:1) to supernatant, shake vigorously (but avoid vortexing!)
- centrifugation at ca. 6000 g for 10 min, room temperature
- collect aqueous phase with cut off pipet tip
- add 2.7 ml isopropanol for precipitation
- centrifugation at ca. 6000 g for 10 min, room temperature
- wash pellet thrice with 2 ml 70% ethanol (pellet should swim in alcohol)
- centrifugation at ca. 6000 g for 10 min and discard liquid
- dry pellet at room temperature over night
- dissolve in 50 µl water with non-acidic pH and transfer into Eppendorf tube.

CTAB-extraction-buffer adjusted to pH 8.0

	Final concentrations	Molecular weight	For 1 L
CTAB	2%	364.46	20 g
NaCl	1.4 M	58.44	81.82 g
EDTA-Na2	20 mM	372.24	7.445 g
Tris	100 mM	121.14	12.114 g

For further analysis by AFLP it is urgently suggested to digest RNA. For PCR amplification followed by fragment sequencing it is not required but improves the precision of DNA quantification by UV (260 nm) measurement.

RNA digestion

- dilute RNase stock solution (100 mg/ml) 1:20 with water and add 1 µl to each sample
- 30 min at 37°C in water bath
- add 49 µl water, 10 µl 3 M Natriumacetate, 300 µl isopropanole and mix thoroughly (no vortexing)
- incubate for 30 min at 4°C
- centrifugation ca. 20,000 g for 20 min at 4°C
- discard liquid
- add 800 µl 70% ethanol (icecold)
- centrifugation ca. 20,000 g for 20 min at 4°C
- discard liquid
- dry pellet at room temperature over night (optional: vacuum concentrator for 5-15 min)
- add 50 µl water (optional: 50 µl TE-buffer).

TE-buffer (pH 8.0-9.0)

	Final concentrations	Molecular weight	For 0.1 L
Tris	10 mM	121.14	0.121 g
EDTA Na2	1 mM	372.24	0.037 g

From the database

Highlights

The first draft genome of the aquatic model plant *Lemna minor* opens the route for future stress physiology research and biotechnological applications

Van Hoeck, A.; Horemans, N.; Monsieurs, P.; Cao, H.X.; Vandenhove, H.; Blust, R.

BIOTECHNOLOGY FOR BIOFUELS 8: Article Number: 188 (2015)

Background: Freshwater duckweed, comprising the smallest, fastest growing and simplest macrophytes has various applications in agriculture, phytoremediation and energy production. Lemna minor, the so-called common duckweed, is a model system of these aquatic plants for ecotoxicological bioassays, genetic transformation tools and industrial applications. Given the ecotoxic relevance and high potential for biomass production, whole-genome information of this cosmopolitan duckweed is needed. The 472 Mbp assembly of the *L. minor* genome (2n = 40; estimated 481 Mbp; 98.1 %) contains 22,382 protein- coding genes and 61.5 % repetitive sequences. The repeat content explains 94.5 % of the genome size difference in comparison with the greater duckweed, Spirodela polyrhiza (2n = 40; 158 Mbp; 19,623 protein-coding genes; and 15.79 % repetitive sequences). Comparison of proteins from other monocot plants, protein ortholog identification, OrthoMCL, suggests 1356 duckweed-specific groups (3367 proteins, 15.0 % total L. minor proteins) and 795 Lemna-specific groups (2897 proteins, 12.9 % total L. minor proteins). Interestingly, proteins involved in biosynthetic processes in response to various stimuli and hydrolase activities are enriched in the Lemna proteome in comparison with the Spirodela proteome. The genome sequence and annotation of *L. minor* protein-coding genes provide new insights in biological understanding and biomass production applications of Lemna species.

Phytoremediation capacity of aquatic plants is associated with the degree of phytochelatin polymerization

Toeroek, A.; Gulyas, Z.; Szalai, G.; Kocsy, G.; Majdik, C.

JOURNAL OF HAZARDOUS MATERIALS 299: 371-378 (2015)

Phytochelatins (PCs) play important role in phytoremediation as heavy metal binding peptides. In the present study, the association between heavy metal removal capacity and phytochelatin synthesis was compared through the examination of three aquatic plants: *Elodea canadensis*, *Salvinia natans* and *Lemna minor*. In case of a Cd treatment, or a Cd treatment combined with Cu and Zn, the highest removal capacity was observed in L. minor. At the same time, *E. canadensis* showed the lowest removal capacity except for Zn. The heavy metal-induced (Cu + Zn + Cd) oxidative stress generated the highest ascorbate level in *L. minor*. Cd in itself or combined with the other two metals induced a 10-15-fold increase in the amount of gamma-glutamylcysteine in *L. minor* while no or smaller changes were observed in the other two species. Correspondingly, the total PC content was 6-8-fold greater in *L. minor*. In addition, PCs with higher degree of polymerization were only observed in *L. minor* (PC4, PC6 and PC7) while PC2 and PC3 occurred in

E. canadensis and *S. natans* only. The correlation analysis indicated that the higher phytoremediation capacity of *L. minor* was associated with the synthesis of PCs and their higher degree of polymerization.

Biotechnology

Impact of density loads on performance of duckweed bioreactor: A potential system for synchronized wastewater treatment and energy biomass production

Verma, R.; Suthar, S.

ENVIRONMENTAL PROGRESS & SUSTAINABLE ENERGY 34: 1596-1604 (2015)

Duckweed *Lemna gibba* has been listed as a promising plant for wastewater treatment and energy biomass production. This study aims to study the efficacy of *L. gibba* based bioreactor in removal of wastewater pollutants loads and energy-rich biomass harvesting under different plant biomass density loads. A total of four density loads: 20% (T1), 40% (T2), 60% (T3), and 80% (T4) of inoculants biomass were used to construct duckweed reactors and changes in wastewater (WW) and duckweed biomass characteristics (growth rate, biochemical composition etc.) were recorded for 21 days. The nutrient load in WW reduced significantly: NO3-N (83-89%), SO42- 30 (85-86%) and total phosphorus (TP) (67-72%) at the end in all experimental set-ups. The reactor with 20% inoculation density showed the maximum WW nutrient removal. The weed biomass yield showed the direct relationship with inoculation density in all experimental set-ups. The yield (dry weight basis) of carbohydrate, starch and protein was: 1.39-1.77 gm(-2)day(-1), 1.10-1.20 gm(-2)day(-1), and 1.9-2.24 gm(-2)day(-1), respectively in different set-ups. The high yield of starch under optimized density load suggests the utility of harvested biomass for bioethanol production. Results thus, suggested that inoculation density directly plays an important role in performance of duckweed reactors in terms of WW treatment and energy biomass synthesis.

Ecology

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

Stirk, W.A.; Kulkarni, M.G.; van Staden, J.

AQUATIC BOTANY 129: 31-34 (2016)

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

Toxicity assessment of aqueous extracts of ash from forest fires

Silva, V; Pereira, J.L.; Campos, I.; Keizer, J.J.; Goncalves, F.; Abrantes, N.

Catena 135: 401-408 (2015)

Wildfires can cause immediate and drastic impacts on the structure and functioning of ecosystems, and there has been an increasing interest in wildfire effects on water chemistry and aquatic biota. Wildfires are increasingly recognized as a diffuse source of contamination of aquatic ecosystems, through the production of deleterious pyrolytic substances and their subsequent transport, mostly attached to ashes. To study the deleterious effects of the ash-laden runoff from burnt areas on water quality, composite ash samples of ashes were collected immediately after a forest fire and then used to prepare aqueous extracts of ash (AEA). The AEA were analyzed with respect to a large group of chemical elements and the sixteen prioritized polycyclic aromatic hydrocarbons (PAHs). Ca, S. Mg, K and Na were found to be the principal elements in the AEA, while only two low molecular weight PAHs (phenanthrene and naphthalene) were present in quantifiable amounts. In parallel, an ecotoxicological screening of the AEA was performed with four standard aquatic species from different functional groups and trophic levels. The AEA was found to induce a statistically significant decrease in the growth of two primary producers *Pseudokirchneriella subcapitata* and *Lemna minor* - and inhibited the luminescence of the bacteria *Vibrio fischeri*. By contrast, AEA did not produce a significant immobilization of *Daphnia magna* suggesting that short-term acute toxicity may be absent at higher trophic levels. Overall, the present results emphasize the role of wildfires as a potential source of diffuse contamination for downstream water bodies, compromising both chemical and ecological conditions. At the same time, this study highlights the need for further research into the complexity of the potentially deleterious ecological effects of wildfires on aquatic communities, with a particular focus on cascading effects along the trophic web.

Feed and Food

Transcriptome analysis of grass carp (*Ctenopharyngodon idella*) fed with animal and plant diets

Li, L.; Liang, X.-F.; He, S.; Sun, J.; Wen, Z.-Y.; He, Y.-H.; Cai, W.-J.; Wang, Y.-P.; Tao, Y.-X.

GENE 574: 371-379 (2015)

Numerous studies have been focused on the replacement of fish meal by other alternative protein sources. However, little is currently known about the molecular mechanism of utilization of diets with different protein sources in fish. Grass carp is a typical herbivorous fish. To elucidate the relationship between gene expression and utilization of animal and plant diets, transcriptome sequencing was performed in grass carp fed with chironomid larvae and duckweed. Grass carp fed with duckweed had significantly higher relative length of gut than those fed with chironomid larvae. 4435 differentially expressed genes were identified between grass carp fed with chironomid larvae and duckweed in brain, liver and gut, involved in cell proliferation and differentiation, appetite control, circadian rhythm, digestion and metabolism pathways. These pathways might play important roles in utilization of diets with different protein sources in grass carp. And the findings could provide a new insight into the replacement of fish meal in artificial diets.

Physiology

Enhanced metabolic and redox activity of vascular aquatic plant *Lemna valdiviana* under polarization in Direct Photosynthetic Plant Fuel Cell

Hubenova, Y.; Mitov, M.

BIOELECTROCHEMISTRY 106: 226-231 (2015)

In this study, duckweed species *Lemna valdiviana* was investigated as a photoautotrophycally grown biocatalyst in recently developed Direct Photosynthetic Plant Fuel Cell. Stable current outputs, reaching maximum of 226 11 mA/m(2), were achieved during the operating period. The electricity production is associated with electrons generated through the light-dependent reactions in the chloroplasts as well as the respiratory processes in the mitochondria and transferred to the anode via endogenous electron shuttle, synthesized by the plants as a specific response to the polarization. In parallel, a considerable increase in the content of proteins (47%) and reserve carbohydrates (44%) of duckweeds grown under polarization conditions was established by means of biochemical analyses. This, combined with the electricity generation, makes the technology a feasible approach for the duckweed farming.

Analysis of electron donors in photosystems in oxygenic photosynthesis by photo-CIDNP MAS NMR

Najdanova, M.; Janssen, G.J.; de Groot, H.J.M.; Matysik, J.; Alia, A.

JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B-BIOLOGY 152: 261-271, Part: B (2015)

Both photosystem I and photosystem II are considerably similar in molecular architecture but they operate at very different electrochemical potentials. The origin of the different redox properties of these RCs is not yet clear. In recent years, insight was gained into the electronic structure of photosynthetic cofactors through the application of photochemically induced dynamic nuclear polarization (photo-CIDNP) with magic-angle spinning NMR (MAS NMR). Non-Boltzmann populated nuclear spin states of the radical pair lead to strongly enhanced signal intensities that allow one to observe the solid-state photo-CIDNP effect from both photosystem I and II from isolated reaction center of spinach (*Spinacia oleracea*) and duckweed (*Spirodela oligorrhiza*) and from the intact cells of the cyanobacterium *Synechocystis* by C-13 and N-15 MAS NMR. This review provides an overview on the photo-CIDNP MAS NMR studies performed on PSI and PSII that provide important ingredients toward reconstruction of the electronic structures of the donors in PSI and PSII.

Phytomedicine

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

Lee, H. J.; Kim, M. H.; Choi, Y. Y.; Kim, E. H.; Hong, J.; Kim, K.; Yang, W. M.

JOURNAL OF ETHNOPHARMACOLOGY 180:12-7 (2016)

Spirodela polyrhiza has been used as a traditional remedy for the treatment of urticarial, acute nephritis, inflammation, as well as skin disease. Atopic dermatitis (AD) is characterized hyperplasia of skin lesion and increase of serum immunoglobulin E (IgE) level. In this study, the topical effects of *S. polyrhiza* (SP) on 2, 4-dinitrochlorobenzene (DNCB)-induced AD mice model were investigated by several experiments.

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

Phytoremediation

Biodegradation of direct blue 129 diazo dye by *Spirodela polyrrhiza*: An artificial neural networks modeling

Movafeghi, A.; Khataee, A. R.; Moradi, Z.; Vafaei, F.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 18: 337-347 (2016)

Phytoremediation potential of the aquatic plant *Spirodela polyrrhiza* was examined for direct blue 129 (DB129) azo dye. The dye removal efficiency was optimized under the variable conditions of the operational parameters including removal time, initial dye concentration, pH, temperature and amount of plant. The study reflected the significantly enhanced dye removal efficiency of *S. polyrrhiza* by increasing the temperature, initial dye concentration and amount of plant. Intriguingly, artificial neural network (ANN) predicted the removal time as the most dominant parameter on DB129 removal efficiency. Furthermore, the effect of dye treatment on some physiologic indices of *S. polyrrhiza* including growth rate, photosynthetic pigments content, lipid peroxidation and antioxidant enzymes were studied. The results revealed a reduction in photosynthetic pigments content and in multiplication of fronds after exposure to dye solution. In contrast, malondialdehyde content as well as catalase (CAT) and peroxidase (POD) activities significantly increased that was probably due to the ability of plant to overcome oxidative stress. As a result of DB129 biodegradation, a number of intermediate compounds were identified by gas chromatography-mass spectroscopy (GC-MS) analysis. Accordingly, the probable degradation pathway of DB129 in *S. polyrrhiza* was postulated.

Removal of fluoride contamination in water by three aquatic plants

Karmakar, S.; Mukherjee, J.; Mukherjee, S.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 18: 222-227 (2016)

Phytoremediation, popularly known as “green technology” has been employed in the present investigation to examine the potential of fluoride removal from water by some aquatic plants. Fluoride contamination in drinking water is very much prevalent in different parts of the world including India. Batch studies were conducted using some aquatic plants e.g., *Pistia stratiotes*, *Eichhornia crassipes*, and *Spirodela polyrhiza* which profusely grow in natural water bodies. The experimental data exhibited that all the above three aquatic floating macrophytes could remove fluoride to some relative degree of efficiency corresponding to initial concentration of fluoride 3, 5, 10, 20mg/l after 10days exposure time. Result showed that at lower concentration level i.e., 3mg/L removal efficiency of *Pistia stratiotes* (19.87%) and *Spirodela polyrhiza* (19.23%) was found to be better as compared to *Eichhornia crassipes* (12.71%). Some of the physiological stress induced parameters such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, total protein, catalase, and peroxidase were also studied to explore relative damage within the cell. A marginal stress was imparted among all the plants for lower concentration values (3mg/L), whereas at 20mg/l, maximum damage was observed.

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

Kalcikova, G.; Zupancic, M.; Jemec, A.; Zgajnar Gotvajn, A.

Chemosphere 147:311-7 (2016)

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

Simultaneous biosorption of the two synthetic dyes, Direct Red 89 and Reactive Green 12 using nonliving macrophyte *L. gibba L.*

Guendouz, S.; Khellaf, N.; Djelal, H.; Ouchefoun, M.

DESALINATION AND WATER TREATMENT 57: 4624-4632 (2016)

This study puts into light the capacity of *Lemna gibba* biomass (LGB) to be used as an effective biosorbent for the simultaneous removal of dyes from contaminated waters. The experiment was carried out at the laboratory scale and focuses on the single and binary biosorption of textile dyes.

Direct Red 89 (DR-89) and Reactive Green 12 (RG-12) two azo dyes were treated with LGB which was selected as a biosorbent. The results showed that very acidic pH value (pH 1) was the optimal value for dye biosorption. When the aqueous system was charged with the two dyes (15mg/L), the biosorption capacity of each individual dye decreased and the sorption capacity of LGB was reduced by 15%. The kinetic modeling results proved that the single biosorption of DR-89 and RG-12 was better accounted for by the pseudo-second-order (R-2>0.96). When the two organic pollutants were simultaneously present in the solution, the kinetic process well fitted the pseudo-second-order and pseudo-first-order models for removing, respectively, DR-89 and RG-12 from the mixture.

Triclosan removal in wetlands constructed with different aquatic plants

Liu, J.; Wang, J.; Zhao, C.; Hay, A.G.; Xie, H.; Zhan, J.

APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 100: 1459-1467 (2016)

Triclosan (TCS) is widely used in consumer products as an antimicrobial agent. Constructed wetlands have the potential for TCS removal, but knowledge about the relative importance of sediment, plants, and microbes is limited. TCS removal performance was investigated in well-operated constructed wetlands planted with three different types of aquatic plants: emergent Cattail (C-T), submerged Hornwort (H-T), and floating *Lemna minor* (L-T). Results showed that the TCS removal efficiencies from water were all greater than 97 %. Maximal TCS adsorption to sediment in the C-T wetland (13.8+/-0.6 ng/g) was significantly lower than in the H-T wetland (21.0+/-0.3 ng/g) or the L-Twetland (21.4+/-0.6 ng/g). The maximal TCS concentrations in plants were 5.7+/-0.2 and 7.2+/-0.5 mu g/g for H-T and L-T, respectively, and it was below the minimal detection limit (MDL) in C-T. Deep 16S rRNA gene sequencing results revealed that C-T wetland had the highest community richness and diversity. Some bacteria, like beta-Proteobacteria, gamma-Proteobacteria, and Bacteroidetes were detected and might have significant correlations with TCS degradation. Overall, with regard to soils, plants, and microorganism, accumulation in sediment and plants in H-T and L-T was high, while in C-T biodegradation likely played an important role.

Removal of mercury (II) from the aquatic environment by phytoremediation

Sitarska, M.; Traczewska, T.; Filyarovskaya, V

DESALINATION AND WATER TREATMENT 57: 1515-1524 (2016)

Phytoremediation may be an alternative to traditional methods of removing heavy metals from the aquatic environment. In order to remove mercury at a concentration of 0.3mg/L from Hoagland medium, two species of pleustophytes were used, namely *Salvinia natans* and *Lemna minor*. Some homogeneous cultures and another one mixed in a weight ratio of 2:1 were used. The physiological condition of the plants was controlled after 7, 14 and 21d by evaluating biomass growth, analyzing the changes in the amount of total protein and assimilation dyes. At the same time, the level of the mercury content in the base and plant tissues was controlled. The increase in biomass during plant exposure to mercury was up to 190% compared to baseline. Accumulation of mercury in plant tissues to 238.34mg/ kg(d.m.), contributed to a significant reduction in its concentration in the medium. The bad condition of the plants led to an attempt to support phytoremediation by micro-organisms taking an active part in the transformation of mercury. For this purpose, epiphytic bacteria, accompanying *S. natans*, which are resistant to mercury at a concentrations of 0.3mg/L, were isolated. Studies indicate the possibility of using the plants *S. natans* and *L. minor* to remove

mercury compounds from the aquatic environment, while providing a basis to determine the principles of design and operation of Lemna ponds, especially when the process is simultaneously stimulated by those bacteria that are resistant to high concentrations of mercury.

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

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

DESALINATION AND WATER TREATMENT 57: 459-467 (2016)

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

Experimental and Kinetic Studies on Penicillin G Adsorption by *Lemna minor*

Balarak, D.; Mostafapour, F.K.; Joghataei, A.

BRITISH JOURNAL OF PHARMACEUTICAL RESEARCH 9, Article Number: UNSP 22820 (2016)

This study examined the ability of dried duckweed (*Lemna minor*) to remove soluble Penicillin G from aqueous solution. Batch experiments were conducted to investigate the effects of pH, contact time, initial Penicillin G concentration, biomass dose and temperature on penicillin G adsorption. Maximum adsorption capacity of the duckweed was 36.18 mg g(-1) when 94.6% of the Penicillin G was removed. The adsorption equilibriums were analyzed by Langmuir, Freundlich, Temkin and BET isotherm models. It was found that he data fitted to Langmuir better than isotherm other models. Batch kinetic experiments showed that the adsorption followed pseudo-second-order kinetic model with correlation coefficients greater than 0.99. The adsorption capacity of penicillin G increased from 31.11 to 41.82 mg/g with increasing the temperature from 20 to 50 degrees C, indicating that the process is endothermic. According to achieved results, it was defined that *Lemna minor* not only was an inexpensive absorbent, but also a quite effective factor in removal of Penicillin G from water and wastewater.

Adsorption and desorption of selenium by two non-living biomasses of aquatic weeds at dynamic conditions

Rodriguez-Martinez, C.E.; Gonzalez-Acevedo, Z.I.; Olguin, M.T.; Frias-Palos, H.

CLEAN TECHNOLOGIES AND ENVIRONMENTAL POLICY 18: 33-44 (2016)

The adsorption and desorption of selenium by non-living biomasses of *Eichhornia crassipes* (Ec) and *Lemna minor* (Lm) at dynamic conditions were evaluated, in terms of: pH, flow direction, mass loading rate, and theoretical speciation. These biomasses are worldwide present in watersheds high in nutrients. The experimental adsorption data were fitted to Thomas Model to obtain the parameters which describe the dynamic process. The Se removal capacity of Ec was 0.3489 A mu g g(-1) and for Lm 0.1855 A mu g g(-1) at pH of 6 and initial selenium concentration of 0.02 mg L-1. For both systems, the vertical flow results are more efficient to remove Se and the horizontal flow is more efficient to recover Se from the Ec packed columns. The highest Se adsorption capacity of non-living biomass of Ec was when the mass loading rate (MLR) is 2.85 mL min(-1) g(-1). For Lm, a MLR of 1.33 mL min(-1) g(-1) was more efficient to adsorb and the less efficient to desorb Se, attributed to its natural swelling physical characteristic and the strong bounding of Se. Both biomasses have the capacity to buffer the pH of the solution, which promotes a species change from selenate () to selenite () during the adsorption process. The data for Ec packed columns are in accordance with the Thomas Model, suggesting that the adsorption process is by ion exchange due to the hydroxide groups naturally present in the non-living biomasses.

Artificial neural network modeling of biotreatment of malachite green by *Spirodela polyrhiza*: Study of plant physiological responses and the dye biodegradation pathway

Torbati, S.

PROCESS SAFETY AND ENVIRONMENTAL PROTECTION 99: 11-19 (2016)

Phytoremediation is an environmental friendly and sustainable means of pollutant remediation through the use of plants. The ability of duckweed (*Spirodela polyrhiza L.*) for decolorization of malachite green was evaluated. Effect of some operational parameters such as initial plant biomass, the reaction time, initial dye concentration, pH and temperature on dye removal efficiency was determined. The importance of each parameter was assessed by artificial neural network (ANN) modeling and the plant initial biomass and pH were found to be the most important factors. The findings indicated that ANN provided reasonable predictive performance (R-2 = 0.98). The metabolic fate of the dye was proposed by identification of 6 intermediate compounds produced during this process by GC -MS technique. Some physiological responses of the plant were studied at 10 and 20 mg/L of the dye. The activities of antioxidant enzymes were increased at high concentration of the contaminant but there was a significant decrease in photosynthetic pigments content at 20 mg/L of malachite green.

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

Bokhari, S.H.; Ahmad, I.; Mahmood-Ul-Hassan, M.; Mohammad, A.

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

Phytoremediation potential of *L. minor* for cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) from two different types of effluent in raw form was evaluated in a glass house experiment using hydroponic studies for a period of 31days. Heavy metals concentration in water and plant sample was analyzed at 3, 10, 17, 24, and 31day. Removal efficiency, metal uptake and bio-concentration factor were also calculated. Effluents were initially analyzed for physical, chemical and

microbiological parameters and results indicated that municipal effluent (ME) was highly contaminated in terms of nutrient and organic load than sewage mixed industrial effluent (SMIE). Results confirmed the accumulation of heavy metals within plant and subsequent decrease in the effluents. Removal efficiency was greater than 80% for all metals and maximum removal was observed for nickel (99%) from SMIE. Accumulation and uptake of lead in dry biomass was significantly higher than other metals. Bio-concentration factors were less than 1000 and maximum BCFs were found for copper (558) and lead (523.1) indicated that plant is a moderate accumulator of both metals. Overall, *L. minor* showed better performance from SMIE and was more effective in extracting lead than other metals.

Research on wastewater phytoremediation using aquatic species for heavy metals phytoextraction and bioaccumulation

Malschi, D.; Oprea, I.C.; Stefanescu, L.; Popita, G.E.; Brahaita, D.I.; Rinba, E.; Kadar, R.

ENVIRONMENTAL ENGINEERING AND MANAGEMENT JOURNAL 14: 2577-2589 (2015)

Phytoremediation tests on heavy metals bioaccumulation were performed during 2012-2014 with aquatic species *Lemna minor*, *Pistia stratiotes*, *Eichhornia crassipes*, *Vallisneria spiralis*, *Hydrilla verticillata*, *Cladophora glomerata* on samples of contaminated waters collected from the toxic pond of waste landfill Somard-Medias, Sibiu County. With the purpose to check the species capacity for heavy metal removal and bioaccumulation, comparative analysis of water samples was performed. Three types of experimental waters for each species have been used: drinking plain water as blank; water collected from the toxic pond of Somard waste landfills; water with 1/2 dilution from the toxic pond of Somard waste landfills. The study was conducted in micro containers with contaminated water using constructed wetlands. The bioaccumulation of heavy metals (Cd, Cu, Ni, Zn, Pb, Fe, Cr) in the green tissue samples (mg/kg dry matter) have been measured after the phytoremediation process. The statistical analysis was performed, based on the determination of microelements with flame atomic absorption spectrometry (ZEEnit 700 Analytik Jena) method. The results have shown that: *L. minor* plants *P. stratiotes* and *E. crassipes* plants were able to extract and to accumulate Cu, Pb, Ni, Fe, Zn, Cr, Cd in their tissues. *V. spiralis* and *H. verticillata* plants were able to extract Cr, Cd. The green algae *C. glomerata* was able to extract Pb, Zn, Cr, Cd. This study has shown that the presence of aquatic species in the toxic pond water have important effects on wastewater phytoremediation in period of two weeks. In order to implement this biotechnology, the tests results have shown a great positive influence of the phytoremediation of contaminated waters using aquatic species in constructed wetlands.

Phytotoxicity

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

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

CHEMOSPHERE 145: 98-105 (2016)

Along with cadmium, lead, mercury and other heavy metals, chromium is an important environmental pollutant, mainly concentrated in areas of intense anthropogenic pressure. The effect

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

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

Cedergreen, N.; Hansen, N.K.K.; Arentoft, B.W.

EUROPEAN JOURNAL OF AGRONOMY 73: 107-117 (2016)

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

Toxicological and chemical assessment of arsenic-contaminated groundwater after electrochemical and advanced oxidation treatments

Radic, S.; Crnojevic, H.; Vujcic, V.; Gajski, G.; Geric, M.; Cvetkovic, Z.; Petra, C.; Garaj-Vrhovac, V.; Orescanin, V.

SCIENCE OF THE TOTAL ENVIRONMENT 543: 147-154 (2016)

Owing to its proven toxicity and mutagenicity, arsenic is regarded a principal pollutant in water used for drinking. The objective of this study was the toxicological and chemical evaluation of groundwater samples obtained from arsenic enriched drinking water wells before and after electrochemical and ozone-UV-H2O2-based advanced oxidation processes (EAOP). For this purpose, acute toxicity test with *Daphnia magna* and chronic toxicity test with *Lemna minor L.* were employed as well as in vitro bioassays using human peripheral blood lymphocytes (HPBLs). Several

oxidative stress parameters were estimated in *L. minor*. Physicochemical analysis showed that EAOP treatment was highly efficient in arsenic but also in ammonia and organic compound removal from contaminated groundwater. Untreated groundwater caused only slight toxicity to HPBLs and D. magna in acute experiments. However, 7-day exposure of *L. minor* to raw groundwater elicited genotoxicity, a significant growth inhibition and oxidative stress injury. The observed genotoxicity and toxicity of raw groundwater samples was almost completely eliminated by EAOP treatment. Generally, the results obtained with *L. minor* were in agreement with those obtained in the chemical analysis suggesting the sensitivity of the model organism in monitoring of arsenic-contaminated groundwater. In parallel to chemical analysis, the implementation of chronic toxicity bioassays in a battery is recommended in the assessment of the toxic and genotoxic potential of such complex mixtures.

Effects of CuO nanoparticles on *Lemna minor*

Song, G.; Hou, W.; Gao, Y. ; Wang, Y.; Lin, L.; Zhang, Z.; Niu, Q.; Ma, R.; Mu, L.; Wang, H.

BOTANICAL STUDIES 57: 3; DOI: 10.1186/s40529-016-0118-x (2016)

Background: Copper dioxide nanoparticles (NPs), which is a kind of important and widely used metal oxide NP, eventually reaches a water body through wastewater and urban runoff. Ecotoxicological studies of this kind of NPs effects on hydrophyte are very limited at present. *Lemna minor* was exposed to media with different concentrations of CuO NPs, bulk CuO, and two times concentration of Cu2+ released from CuO NPs in culture media. The changes in plant growth, chlorophyll content, antioxidant defense enzyme activities [i.e., peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) activities], and malondialdehyde (MDA) content were measured in the present study. The particle size of CuO NPs and the zeta potential of CuO NPs and bulk CuO in the culture media were also analyzed to complementally evaluate their toxicity on duckweed. Results showed that CuO NPs inhibited the plant growth at lower concentration than bulk CuO. *L. minor* roots were easily broken in CuO NPs media under the experimental condition, and the inhibition occurred only partly because CuO NPs released Cu2+ in the culture media. The POD, SOD, and CAT activities of L. minor increased when the plants were exposed to CuO NPs, bulk CuO NPs and two times the concentration of Cu2+ released from CuO NPs in culture media, but the increase of these enzymes were the highest in CuO NPs media among the three kinds of materials. The MDA content was significantly increased compared with that of the control from 50 mg L-1 CuO NP concentration in culture media. CuO NPs has more toxicity on *L. minor* compared with that of bulk CuO, and the inhibition occurred only partly because released Cu2+ in the culture media. The plant accumulated more reactive oxygen species in the CuO NP media than in the same concentration of bulk CuO. The plant cell encountered serious damage when the CuO NP concentration reached 50 mg L-1 in culture media. The toxicology of CuO NP on hydrophytes must be considered because that hydrophytes are the basic of aquatic ecosystem.

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

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

JOURNAL OF HAZARDOUS MATERIALS 302: 351-361 (2016)

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

Phytotoxicity of wastewater-born micropollutants - Characterisation of three antimycotics and a cationic surfactant

Richter, E.; Roller, E.; Kunkel, U.; Ternes, T.A.; Coors, A.

ENVIRONMENTAL POLLUTION 208: 512-522 Part: B (2016)

Sewage sludge applied to soil may be a valuable fertiliser but can also introduce poorly degradable and highly adsorptive wastewater-born residues of pharmaceuticals and personal care products (PPCPs) to the soil, posing a potential risk to the receiving environment Three azole antimycotics (climbazole, ketoconazole and fluconazole), and one quaternary ammonium compound (benzyldimethyldodecylammonium chloride, BDDA) that are frequently detected in municipal sewage sludge and/or treated wastewater were therefore characterised in their toxicity toward terrestrial (*Brassica napus*) and aquatic (*Lemna minor*) plants. Fluconazole and climbazole showed the greatest toxicity to *B. napus*, while toxicity of ketoconazole and BDDA was by one to two orders of magnitude lower. Sludge amendment to soil at an agriculturally realistic rate of 5 t/ha significantly reduced the bioconcentration of BDDA in *B. napus* shoots compared to tests without sludge amendment, although not significantly reducing phytotoxicity. Ketoconazole, fluconazole and BDDA proved to be very toxic to *L. minor* with median effective concentrations ranging from 55.7 mu g/L. to 969 mu g/L. In aquatic as well as terrestrial plants, the investigated azoles exhibited growth-retarding symptoms presumably related to an interference with phytohormone synthesis as known for structurally similar fungicides used in agriculture. While all four substances exhibited considerable phytotoxicity, the effective concentrations were at least one order of magnitude higher than concentrations measured in sewage sludge and effluent. Based on preliminary hazard quotients, BDDA and climbazole appeared to be of greater environmental concern than the two pharmaceuticals fluconazole and ketoconazole.

How TK-TD and Population Models for Aquatic Macrophytes Could Support the Risk Assessment for Plant Protection Products

Hommen, U.; Schmitt, W.; Heine, S.; Brock, T.C.M.; Duquesne, S.; Manson, P.; Meregalli, G.; Ochoa-Acuna, H.; van Vliet, P.; Arts, G.

INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT 12: 82-95 (2016)

This case study of the Society of Environmental Toxicology and Chemistry (SETAC) workshop MODELINK demonstrates the potential use of mechanistic effects models for macrophytes to extrapolate from effects of a plant protection product observed in laboratory tests to effects resulting from dynamic exposure on macrophyte populations in edge-of-field water bodies. A standard European Union (EU) risk assessment for an example herbicide based on macrophyte laboratory tests indicated risks for several exposure scenarios. Three of these scenarios are further analyzed using effect models for 2 aquatic macrophytes, the free-floating standard test species *Lemna sp.*, and the sediment-rooted submerged additional standard test species *Myriophyllum spicatum*. Both models include a toxicokinetic (TK) part, describing uptake and elimination of the toxicant, a toxicodynamic (TD) part, describing the internal concentration-response function for growth inhibition, and a description of biomass growth as a function of environmental factors to allow simulating seasonal dynamics. The TK-TD models are calibrated and tested using laboratory tests, whereas the growth models were assumed to be fit for purpose based on comparisons of predictions with typical growth patterns observed in the field. For the risk assessment, biomass dynamics are predicted for the control situation and for several exposure levels. Based on specific protection goals for macrophytes, preliminary example decision criteria are suggested for evaluating the model outputs. The models refined the risk indicated by lower tier testing for 2 exposure scenarios, while confirming the risk associated for the third. Uncertainties related to the experimental and the modeling approaches and their application in the risk assessment are discussed. Based on this case study and the assumption that the models prove suitable for risk assessment once fully evaluated, we recommend that 1) ecological scenarios be developed that are also linked to the exposure scenarios, and 2) quantitative protection goals be set to facilitate the interpretation of model results for risk assessment.

Influence of nutrient medium composition on uranium toxicity and choice of the most sensitive growth related endpoint in *Lemna minor*

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JOURNAL OF ENVIRONMENTAL RADIOACTIVITY 151: 427-437 (2016), Part: 2, Special Issue: SI

Uranium (U) toxicity is known to be highly dependent on U speciation and bioavailability. To assess the impact of uranium on plants, a growth inhibition test was set up in the freshwater macrophyte *Lemna minor*. First growth media with different compositions were tested in order to find a medium fit for testing U toxicity in *L. minor*. Following arguments were used for medium selection: the ability to sustain *L. minor* growth, a high solubility of U in the medium and a high percentage of the more toxic U-species namely UO22+. Based on these selection criteria a with a low phosphate concentration of 0.5 mg L-1 and supplemented with 5 mM MES (2-(N-morpholino)ethanesulfonic acid) to ensure pH stability was chosen. This medium also showed highest U toxicity compared to the other tested media. Subsequently a full dose response curve for U was established by exposing *L. minor* plants to U concentrations ranging from 0.05 mu M up to 150 mu M for 7 days. Uranium was shown to adversely affect growth of *L. minor* in a dose dependent manner with EC10, EC30 and EC50 values ranging between 1.6 and 4.8 mu M, 7.7-16.4 mu M and 19.4-37.2 mu M U, respectively, depending on the growth endpoint. Four different growth related endpoints were tested: frond area, frond number, fresh weight and dry weight. Although differences in relative growth rates and associated ECx-values calculated on different endpoints are small (maximal twofold difference), frond area is recommended to be used to measure U-induced growth effects as it is a sensitive growth endpoint and easy to measure in vivo allowing for measurements over time.

A comparative study of electrochemical degradation of imidazolium and pyridinium ionic liquids: A reaction pathway and ecotoxicity evaluation

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SEPARATION AND PURIFICATION TECHNOLOGY 156: 522-534 (2015)

The investigation focused on the electrochemical oxidation of imidazolium and pyridinium ionic liquids (ILs), 1-butyl-3-methyl-imidazolium chloride (IM14 Cl), 1-hexyl-3-methyl-imidazolium chloride (IM16 Cl), 1-methyl-3-(2-phenylethyl)-imidazolium chloride (IM1-2Ph Cl), 1-butyl-4-methylpyridinium chloride (Py4-4Me Cl), and 1-butyl-4-(dimethylamino)pyridinium chloride (Py4-4NMe2 Cl) in an aqueous solution at the boron-doped diamond (BDD) electrode. The study demonstrated that the lowest degradation efficiency occurs at alkaline pH, while an increase in temperature has only a slight effect on the electrochemical oxidation process. The intermediate products, identified by LC-(ESI)/MS and GC-MS methods, suggest that the (OH)-O-center dot and O2(center dot-) radicals take part in the decomposition of the investigated ILs. The attack of (OH)-O-center dot at first led to the hydroxylation of ILs followed by the oxidative opening of imidazolium or pyridinium ring. After a 3 h electrolysis, the decomposition of pyridinium salts was more advanced compared to imidazolium salts. Finally, the toxicity of untreated ILs solutions and their effluents after the electrochemical treatment was assessed from the measurements of growth inhibition in *Scenedesmus vacuolatus* and *Lemna minor*. The investigated ILs were more toxic to *S. vacuolatus* than *L. minor*. In general, the toxicity values decreased after the electrochemical treatment. However, in the case of IM14Cl/NaCl mixture, its post-electrolytic toxicity strongly increased.

Assessment of acrylamide toxicity using a battery of standardised bioassays

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ARHIV ZA HIGIJENU RADA I TOKSIKOLOGIJU-ARCHIVES OF INDUSTRIAL HYGIENE AND TOXICOLOGY 66: 315-321 (2015)

Acrylamide is a monomer widely used as an intermediate in the production of organic chemicals, e.g. polyacrylamides (PAMs). Since PAMs are low cost chemicals with applications in various industries and waste-and drinking water treatment, a certain amount of non-polymerised acrylamide is expected to end up in waterways. PAMs are non-toxic but acrylamide induces neurotoxic effects in humans and genotoxic, reproductive, and carcinogenic effects in laboratory animals. In order to evaluate the effect of acrylamide on freshwater organisms, bioassays were conducted on four species: algae *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata*, duckweed *Lemna minor* and water flea *Daphnia magna* according to ISO (International Organization for Standardisation) standardised methods. This approach ensures the evaluation of acrylamide toxicity on organisms with different levels of organisation and the comparability of results, and it examines the value of using a battery of low-cost standardised bioassays in the monitoring of pollution and contamination of aquatic ecosystems. These results showed that EC50 values were lower for *Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata* than for *Daphnia magna* and *Lemna minor*, which suggests an increased sensitivity of algae to acrylamide. According to the toxic unit approach, the values estimated by the *Lemna minor* and *Daphnia magna* bioassays, classify acrylamide as slightly toxic (TU=0-1; Class 1). The results obtained from algal bioassays

(*Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata*) revealed the toxic effect of acrylamide (TU=1-10; Class 2) on these organisms.

Resistance to mercury epiphytic bacteria *Lemna minor*

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Architectur Civil Engeneering Envirioment 8: 89-92 (2015)

The developing of new alternative methods of purifying the environment of mercury is extremely important in terms of growing restrictions of legal norms issued by the European Union. One of such methods is phytoremediation. The obtained results of the application *Salvinia natans* to remediate groundwater contaminated with mercury and the stimulation of this process through the active strains of epiphytic has interest for analogous effects phytoremediation by *Lemna minor*. Plants cavitation in physiological solution, corns on solid ground of morphological part of plants, seed stocks of homogenised tissue were used to guarantee the effectiveness of the isolation of epiphytic bacteria.

Manganese toxicity to tropical freshwater species in low hardness water

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ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 34: 2856-2863 (2015)

Elevated manganese (Mn) is a common contaminant issue for mine water discharges, and previous studies have reported that its toxicity is ameliorated by H+, Ca2+, and Mg2+ ions. In the present study, the toxicity of Mn was assessed in a high risk scenario, that is, the slightly acidic, soft waters of Magela Creek, Kakadu National Park, Northern Territory, Australia. Toxicity estimates were derived for 6 tropical freshwater species (*Chlorella sp.*, *Lemna aequinoctialis*, *Amerianna cumingi*, *Moinodaphnia macleayi*, *Hydra viridissima*, and *Mogurnda mogurnda*). Low effect chronic inhibition concentration (IC10) and acute lethal concentration (LC05) values ranged between 140gL(-1) and 80000gL(-1), with 3 of the species tested (*M. macleayi*, *A. cumingi*, and *H. viridissima*) being more sensitive to Mn than all but 1 species in the international literature (*Hyalella azteca*). A loss of Mn was observed on the final day for 2 of the *H. viridissima* toxicity tests, which may be a result of the complex speciation of Mn and biological oxidation. International data from toxicity tests conducted in natural water with a similar physicochemistry to Magela Creek water were combined with the present study's data to increase the sample size to produce a more reliable species sensitivity distribution. A 99% protection guideline value of 73 gL(-1) (33-466gL(-1)) was derived; the low value of this guideline value reflects the higher toxicity of Mn in slightly acidic soft waters.

Ecotoxicological Effects of an Arsenic Remediation Method on Three Freshwater Organisms-*Lemna disperma*, *Chlorella sp* CE-35 and *Ceriodaphnia cf. dubia*

Rahman, M.A.; Hogan, B.; Duncan, E. ; Doyle, C.; Rahman, M.M.; Nguyen, T.V.; Lim, R.P.; Maher, W.; Naidu, R.; Krassoi, R.

WATER AIR AND SOIL POLLUTION 226, Issue: 12, article number 441 (2015)

Chemical methods have been used for the remediation of arsenic (As)-contaminated water; however, ecological consequences of these methods have not been properly addressed. The present study evaluated the effects of the Fe-oxide-coated sand (IOCS) remediation method on As toxicity to freshwater organisms (*Lemna disperma*, *Chlorella sp.* CE-35, and *Ceriodaphnia cf. dubia*). The As removal efficiency by IOCS decreased substantially with time. The IOCS remediation method was less effective at suppressing the toxicity of As-V than As-III to *L. disperma* but was highly effective in reducing both the As-III and As-V toxicity to *C. cf. dubia*. The growth of *Chlorella sp.* was significantly higher ($p<0.05$) in remediated and pre-remediated water than in controls (non-As-contaminated filtered Colo River water) for As-III, while the opposite was observed for As-V, indicating that As-V is more toxic than As-III to this microalga. Although the IOCS can efficiently remove As from contaminated water, residual As and other constituents (e.g. Fe, nitrate) in the remediated water had a significant effect on freshwater organisms.

Application of simple and low-cost toxicity tests for ecotoxicological assessment of industrial wastewaters

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ENVIRONMENTAL TECHNOLOGY 36: 2825-2834 (2015)

The objective of this study was to identify and to apply appropriate biotests having the advantages of being highly sensitive, easy to run, relatively inexpensive and able to substitute fish toxicity tests due to ethical reasons of animal welfare. To perform an ecotoxicological assessment of industrial wastewaters, different microbiotests were conducted to substitute the fish toxicity test with *Lebistes reticulatus* through *Vibrio fischeri*, *Thamnocephalus platyurus*, *Daphnia magna*, *Lemna minor* and *Lepidium sativum* representing different trophic levels in the aquatic and terrestrial ecosystems. Also, Algaltox F-TM with *Pseudokirchneriella subcapitata* and Protox F-TM with *Tetrahymena thermophila* tests were carried out. However, they could not be applied successfully for the wastewater samples. Wastewater samples from seven different industrial zones comprising different industries were subjected to characterization through measuring their physical-chemical parameters and their toxicity versus the above-mentioned organisms. *T. platyurus*, *D. magna* and *L. reticulatus* were the most sensitive test organisms investigated for the wastewaters. Considering toxic unit values, generally wastewater samples were toxic according to Thamnotox F-TM, Daphtox F-TM and fish toxicity tests. As an important outcome, it was concluded that Daphtox F-TM and Thamnotox F-TM could be a good alternative for the fish toxicity test, which is so far the sole toxicity test accepted by the Turkish Water Pollution Control Regulation.

Investigations on sediment toxicity of German rivers applying a standardized bioassay battery

Hafner, C.; Gartiser, S.; Garcia-Kaeufer, M.; Schiwy, S.; Hercher, C.; Meyer, W.; Achten, C.; Larsson, M.; Engwall, M.; Keiter, S.

ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 22: 16358-16370 (2015)

River sediments may contain a huge variety of environmental contaminants and play a key role in the ecological status of aquatic ecosystems. Contaminants adsorbed to sediments and suspended solids may contribute directly or after remobilization to an adverse ecological and chemical status of surface water. In this subproject of the joint research project DanTox, acetonic Soxhlet extracts from three German river sediments from the River Rhine (Altrip and Ehrenbreitstein with moderate

contamination) and River Elbe (Veringkanal Hamburg heavily contaminated) were prepared and redissolved in dimethyl sulfoxide (DMSO). These extracts were analyzed with a standard bioassay battery with organisms from different trophic levels (bacteria, algae, *Daphnia*, fish) as well as in the Ames test and the umuC test for bacterial mutagenicity and genotoxicity according to the respective OECD and ISO guidelines. In total, 0.01 % (standard) up to 0.25 % (only fish embryo test) of the DMSO sediment extract was dosed to the test systems resulting in maximum sediment equivalent concentrations (SEQ) of 2 up to 50 g l(-1). The sediment of Veringkanal near Hamburg harbor was significantly more toxic in most tests compared to the sediment extracts from Altrip and Ehrenbreitstein from the River Rhine. The most toxic effect found for Veringkanal was in the algae test with an ErC50 (72 h) of 0.00226 g l(-1) SEQ. Ehrenbreitstein and Altrip samples were about factor 1,000 less toxic. In the *Daphnia*, *Lemna*, and acute fish toxicity tests, no toxicity at all was found at 2 g l(-1) SEQ. corresponding to 0.01 % DMSO. Only when increasing the DMSO concentration the fish embryo test showed a 22-fold higher toxicity for Veringkanal than for Ehrenbreitstein and Altrip samples, while the toxicity difference was less evident for the *Daphnia* test due to the overlaying solvent toxicity above 0.05 % dimethyl sulfoxide (DMSO). The higher toxicities observed with the Veringkanal sample are supported by the PAH and PCB concentrations analyzed in the sediments. The sediment extracts of Altrip andVeringkanal were mutagenic in the Ames tester strain TA98 with metabolic activation (S9mix). The findings allow a better ecotoxicological characterization of the sediments extensively analyzed in all subprojects of the DanTox project (e. g., Garcia-Kaeufer et al. Environ Sci Pollut Res. doi: 10.1007/s11356-014-3894-4, 2014; Schiwy et al. Environ Sci Pollut Res. doi: 10.1007/s11356-014-31850, 2014; Hollert and Keiter 2015). In the absence of agreed limit values for sediment extracts in standard tests, further data with unpolluted reference sediments are required for a quantitative risk assessment of the investigated polluted sediments.

Acute Toxicity Assessment of Reactive Red 120 to Certain Aquatic Organisms

Darsana, R.; Chandrasehar, G.; Deepa, V.; Gowthami, Y.; Chitrikha, T.; Ayyappan, S.; Goparaju, A.

BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 95: 582-587 (2015)

Laboratory experiments were conducted to evaluate the acute toxicity of a widely used textile dye namely Reactive Red 120 (RR 120) on certain aquatic species such as *Pseudokirchneriella subcapitata* (green alga), *Lemna gibba* (duckweed), *Daphnia magna* (water flea) and *Oncorhynchus mykiss* (Rainbow trout). All experiments were performed as per the OECD Guidelines for Testing of Chemicals. The toxicity end points of EC50, LC50, NOEC and LOEC for RR 120 were determined with 95 % confidence limits using TOX STAT version 3.5. The EC50 of RR 120 for green alga, duckweed and water flea are > 100.00, 64.34, 10.40 mg L-1, respectively and LC50 for Rainbow trout is 78.84 mg L-1. Based on the results, the test item RR 120 could be classified as non-toxic to green alga, harmful to duck weed and Rainbow trout, toxic to water flea.

Links for Further Reading

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

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

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

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

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

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