September 2014, letter #6

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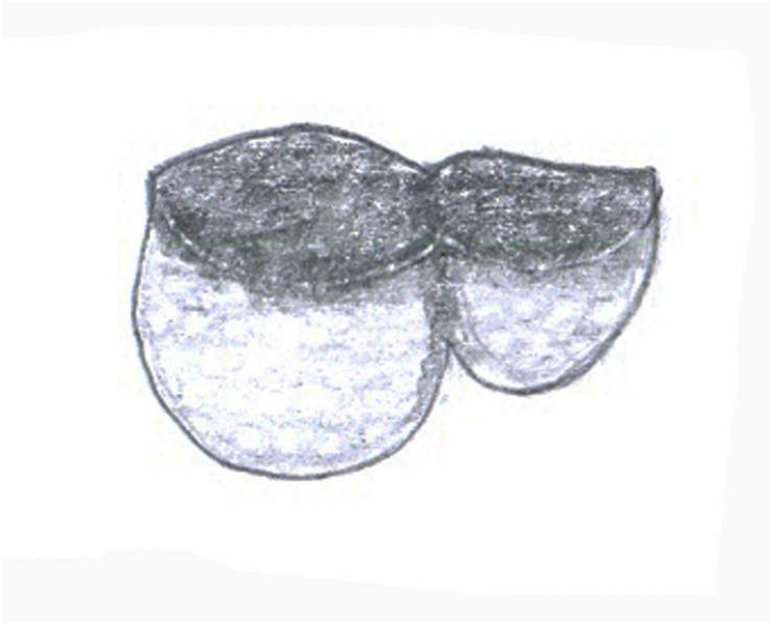
ISCDRA



Wolffia arrhiza (L.) Horkel ex Wimm., mother frond (left) and daughter frond (right). Plants of the genus Wolffia (11 species, distributed worldwide) are being used for human consumption for many generations now, e.g. in Thailand, Laos, or Cambodia. W. arrhiza is often reported to be used as human food but a very close relative, W. globosa, is the most common species used in these countries. These plants are rich in proteins. Drawing by Dr. K. Sowjanya Sree, India.

International Lemna Association's 75th Meeting

By ILA Exec. Director Tamra Fakhoorian

Our first of many ILA Round Table online meetings was held in May of 2012 with several countries being represented. All participants had high energy around business models for commercializing duckweed. Everyone knew they were making history. Rarely did a meeting end on time- there was too much to share and discuss!

Seventy-five meetings later, that energy is just as palpable and meetings are still just as difficult to end on time given the vibrant conversations. It is inspiring to witness the level of expertise being shared just as freely now as back in 2012. It has been exciting to witness company start-ups and partnerships form as a result of the ILA Round Table. Everyone continues to offer their expertise, support, and encouragement for one another. We have become family.

During the past two years, we've made small changes to our meeting structure. The meetings are now open to the entire duckweed community four times a year. All newcomers to the ILA are invited to sit in on a meeting as guests to understand the opportunities in the fledgling industry. We now host featured speakers to present at every other meeting with topics ranging from marketing duckweed products to overcoming production roadblocks. Many of these presentations are recorded and posted on the ILA website.

It has been an honor to moderate these ground-breaking meetings and an even greater honor to call all who participate- friends and fellow duckweed visionaries.

Here's to the next seventy-five meetings! May they continue to be rewarding for our members and the overall mission of the ILA- that of developing commercial production of duckweed for renewable, sustainable products for a hungry and increasingly fresh water limited world.

Inquiries about the ILA should be directed to Tamra Fakhoorian Exec. Director ILA (tamraf9@gmail.com) or http://www.internationallemnaassociation.org

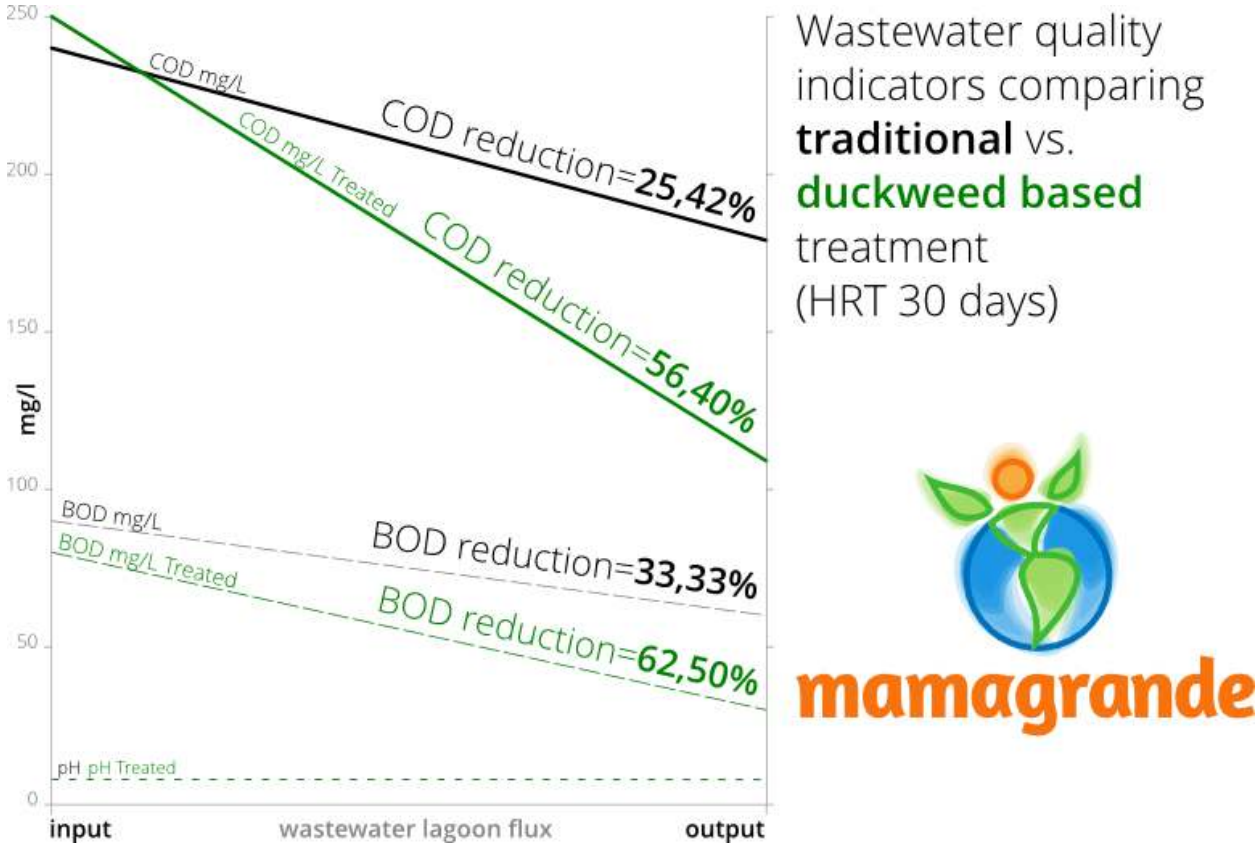
Official News: Duckweed in Argentine Wastewater Treatment

Duckweed-based wastewater remediation process shows BOD/COD 200% performance

MamaGrande is a social enterprise from Argentina that developed an integrated wastewater to biopolymer process based on bacteria/duckweed synergy. Its pilot plant in Totoras town (11.000 inhabitants) is treating ~750m3/day. But, to what extent is the water treated?

To compare hard data of the duckweed based process with the traditional process, MamaGrande paired with the ENRESS (Regulatory Entity of Sanitary Services, Santa Fe province, Argentina), the official Governmental entity responsible for water quality.

The engineers from ENRESS take WWT lagoons water quality regular measurements according to the international parameters: BOD, COD, pathogens, etc., with the following results:



Treatment	Indicator	input (mg/l)	output (mg/l)	Reduction (%)
Traditional	BOD	90	60	33,33 %
Traditional	COD	240	179	25,42 %
Bacteria+Duckweed	BOD	80	30	62,50 %
Bacteria+Duckweed	COD	250	109	56,40 %

These are the first reports that we know of real scale, ongoing, field proven duckweed based WWT in Latin America coming from official Governmental Institutions.

We will continue advancing with the pilot and scaling up.

Requests for information or inquiries can be sent to info@mamagrande.org.

Student Researcher Spotlight: Pooja Tendulkar from India

Fergusson College, Pune, India

I have had keen interest for research in biology since my high school days. This made me to opt for Biotechnology for my graduation course after high school. One of the courses, Environmental Biotechnology, sparked my curiosity in the field of second generation biofuels as well as biomass energy.

I applied for the PICC (Pune Inter-Collegiate Consortium) funding fellowship which is granted by my college for upcoming young researchers and I was selected for an interview. I chose to research biomass energy as a niche subject that I could really make a difference in and stand out of the crowd with. As expected, the interview panel was not only drawn to the fullest to my topic, but was also engaged in a continuous exchange of ideas over it. I was granted the fellowship.

I then began seeking a mentor to help me with my project and soon became

acquainted with Mr. Prasanna Jogdeo and his duckweed research. He provided me with the basic knowledge of duckweed, but made me scratch my head whenever I raised a question. I found myself searching extensively for all kinds of information I could get my hands on and went through more than 50 research papers which dealt with numerous potentials of duckweed. The work matrix was formulated knowing the current issues of water pollution as well as fuel price hikes. We finalized the title of "Phytoremediation, Bioethanol Production and its Use as Animal Feed." The project work was finally submitted.

After two amazing years of successful research on duckweed, I look forward to taking this research on to the next level for my Masters' dissertation work, allowing me to further explore opportunities in utilizing this abundant, unused gift of nature.

During the course of my research, I have become aware of ever-rising pollution levels due to industrial effluent discharge and recognize its damaging effects on living beings as far beyond our imagination. The worst part of this is that the repercussions of our ill deeds will be paid by forthcoming generations. This made me develop a study on hyper accumulation of duckweed.

However, biofuel engineering of duckweed is the concept which fascinates me the most. Duckweed has eminent potential as a second generation biofuel. I am really keen on focusing upon its pyrolysis as well as trans-esterification. One day as I see it, not far from today, it will reduce our world dependence on petroleum, just as ethanol has. The search for a better fuel has always been so exciting. I would really love to work upon it if given a chance. I am very conscious about the originality and authenticity of the work I do, and enjoy my journey to the future with endless possibilities!

Contact: Pooja Tendulkar <poojatendulkar007@gmail.com>

From the Data Base

Note: In this edition, many of the papers are cited (Early View, eVersion ahead of print) which will be published in the Special Issue of PLANT BIOLOGY

*"**After the genome sequencing of duckweed - how to proceed with research on the fastest growing Angiosperm?**" edited by Klaus-J. Appenroth, Daniel J. Crawford and Donald H. Les. This is only one reason why this time this part of the NewsLetter is so extended . The Special Issue is dedicated to the late Elias Landolt. The hard copy will be available in January 2015. We will inform you as soon as this issue is available.*

Phytoremediation of anatoxin a by aquatic macrophyte Lemna trisulca L.

Chemosphere. 2014 Oct;112:305-10. doi: 10.1016/j.chemosphere.2014.04.064

Kaminski A, Bober B, Chrapusta E, Bialczyk J

The neurotoxin anatoxin-a (ANTX-a), one of the most common cyanotoxin, poses a health risk to people and can be lethal to aquatic organisms. This paper presents results on its bioremediation by the aquatic macrophyte Lemna trisulca. We show that the plant is resistant to the harmful impact of toxin and is capable of removing ANTX-a from water. Some of the ANTX-a concentrations which were used in our experiments were much higher than those found in natural conditions. The exposition of L. trisulca to 2.5 μg ANTX-a/mL did not affect its biomass accumulation within 24 d. Significant decreases in biomass content by 21% and 30% were demonstrated in samples cultivated in media containing 12.5 μg ANTX-a/mL after 18 and 24 day of experiment, respectively. One gram of fresh weight (f.w.) of L. trisulca cultured for 14 d in the media containing 50 μg ANTX-a removed 95% of the initial toxin concentration; for media with 250 μg ANTX-a, 86% was removed. In tests of ANTX-a binding stability and degradation we transferred the macrophyte to fresh media without added toxin; within 14 d the content of accumulated ANTX-a in the macrophyte decreased by 76% (from initial 19.3 μg ANTX-a/gf.w.), 71% (from 37.3 μg ANTX-a/g f.w.) and 47% (from 63.7 μg ANTX-a/g f.w.). The quantity of ANTX-a released to media was minimal: from 3.5% to 5.1% of the initial bioaccumulated value. The data show that part of the ANTX-a was degraded. Mass spectra analyses did not indicate transformation of ANTX-a to already known forms. These findings suggest that L. trisulca has much potential as a phytoremediation

agent for stabilization of aquatic environments.

Chemical characterisation and analysis of the cell wall polysaccharides of duckweed (Lemna minor)

Carbohydr Polym. 2014 Oct 13;111:410-8. doi: 10.1016/j.carbpol.2014.04.079

Zhao X, Moates GK, Wellner N, Collins SR, Coleman MJ, Waldron KW

Duckweed is potentially an ideal biofuel feedstock due to its high proportion of cellulose and starch and low lignin content. However, there is little detailed information on the composition and structure of duckweed cell walls relevant to optimising the conversion of duckweed biomass to ethanol and other biorefinery products. This study reports that, for the variety and batch evaluated, carbohydrates constitute 51.2% (w/w) of dry matter while starch accounts for 19.9%. This study, for the first time, analyses duckweed cell wall composition through a detailed sequential extraction. The cell wall is rich in cellulose and also contains 20.3% pectin comprising galacturonan, xylogalacturonan, rhamnogalacturonan; 3.5% hemicellulose comprising xyloglucan and xylan, and 0.03% phenolics. In addition, essential fatty acids (0.6%, α-linolenic and linoleic/linoelaidic acid) and p-coumaric acid (0.015%) respectively are the most abundant fatty acids and phenolics in whole duckweed.

Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: a pilot-scale comparison with water hyacinth

Bioresour Technol. 2014, 163:82-91

Zhao Y, Fang Y, Jin Y, Huang J, Bao S, Fu T, He Z, Wang F, Zhao H

The application potential of duckweed (Lemna japonica 0234) and water hyacinth (Eichhornia crassipes) were compared in two pilot-scale wastewater treatment systems for more than one year. The results indicated duckweed had the same total nitrogen (TN) recovery rate as water hyacinth (0.4 g/m(2)/d) and a slightly lower total phosphorus (TP) recovery rate (approximately 0.1g/m(2)/d) even though its biomass production was half that of water hyacinth. The higher content of crude protein (33.34%), amino acids (25.80%), starch (40.19%), phosphorus (1.24%), flavonoids (2.91%) and lower fiber content provided duckweed with more advantages in resource utilization. Additionally, microbial community discovered by 454 pyrosequencing indicated that less nitrifying bacteria and more nitrogen-fixing bacteria in rhizosphere of duckweed provided it with higher nitrogen recovery efficiency (60%) than water

hyacinth (47%). Under the presented condition, duckweed has more application advantages than water hyacinth because it more effectively converted the wastewater nutrients into valuable biomass.

A marriage of convenience; a simple food chain comprised of Lemna minor (L.) and Gammarus pulex (L.) to study the dietary transfer of zinc

Plant Biol. 2014 Apr 14. doi: 10.1111/plb.12179

Lahive E, O'Halloran J, Jansen MA

Macrophytes contribute significantly to the cycling of metals in aquatic systems, through accumulation during growth and release during herbivory or decomposition. Accumulation of high levels of metals has been extensively documented in Lemnaceae (duckweeds). However, the degree of trophic transfer of metals from Lemnaceae to secondary consumers remains poorly understood. This study demonstrates that zinc accumulated in Lemna minor is bioavailable to the herbivore consumer Gammarus pulex. Overall, the higher the zinc content of L. minor, the more zinc accumulated in G. pulex. Accumulation in G. pulex was such that mortality occurred when they were fed high zinc-containing L. minor. Yet, the percentage of consumed zinc retained by G. pulex actually decreased with higher zinc concentrations in L. minor. We hypothesise that this decrease reflects internal zinc metabolism, including a shift from soluble to covalently bound zinc in high zinc-containing L. minor. Consistently, relatively more zinc is lost through depuration when G. pulex is fed L. minor with high zinc content. The developed Lemna-Gammarus system is simple, easily manipulated, and sensitive enough for changes in plant zinc metabolism to be reflected in metal accumulation by the herbivore, and therefore suitable to study ecologically relevant metal cycling in aquatic ecosystems.

Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant Spirodela polyrhiza

Environ Toxicol Chem. 2014, 33:1398-405.

Jiang HS, Qiu XN, Li GB, Li W, Yin LY

Erratum in Environ Toxicol Chem. 2014, 33:1914.

Silver nanoparticles (AgNPs) are widely used commercially because of their antibacterial properties. Oxidative stress is known to be involved in the toxicity of

AgNPs to bacteria, animals, and algae. The authors used Spirodela polyrhiza to investigate whether AgNPs can induce oxidative stress in higher plants. Results showed that there was a dose-dependent increase in levels of reactive oxygen species, superoxide dismutase and peroxidase activity, and the antioxidant glutathione content in 6-nm AgNP treatments. Catalase activity and malondialdehyde content in 6-nm AgNP treatments was significantly higher than the control at silver concentrations of 5 mg L(-1) . Superoxide dismutase and catalase activity and antioxidant glutathione and malondialdehyde content were not significantly different at 10 mg L(-1) of AgNPs (6 nm and 20 nm). Treatment with 20 μg L(-1) Ag(+) (the amount almost equal to 10 mg L(-1) AgNPs released) did not change the reactive oxygen species level or antioxidant enzymes activity. Micron-sized Ag particles had no effect on S. polyrhiza. Transmission electron microscopy showed that, compared with the control, chloroplasts in S. polyrhiza treated with 6-nm and 20-nm AgNPs accumulated starch grains and had reduced intergranal thylakoids. These results clearly indicate that AgNPs are able to cause oxidative stress and affect the chloroplast structure and function of S. polyrhiza, and this effect was not caused by Ag(+) released from particles.

The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater

Bioresour Technol. 2014, 167:383-9.

Zhao Z, Shi H, Liu Y, Zhao H, Su H, Wang M, Zhao Y

The effect of temperature, light intensity, nitrogen and phosphorus concentrations on the biomass and starch content of duckweed (Landoltia punctata OT, Lemna minor OT) in monoculture and mixture were assessed. Low light intensity promoted more starch accumulation in mixture than in monoculture. The duckweed in mixture had higher biomass and nutrient removal efficiency than those in monoculture in swine wastewater. Moreover, the ability of L. punctata C3, L. minor C2, Spirodela polyrhiza C1 and their mixtures to recovery nutrients and their biomass were analyzed. Results showed that L. minor C2 had the highest N and P content, while L. punctata C3 had the highest starch content, and the mixture of L. punctata C3 and L. minor C2 had the greatest nutrient removal rate and the highest biomass. Compared with L. punctata C3 and L. minor C2 in monoculture, their biomass in mixture increased by 17.0% and 39.8%, respectively.

Characterisation of circadian rhythms of various duckweeds

Plant Biol 2014 Jun 18. doi: 10.1111/plb.12202

Muranaka T, Okada M, Yomo J, Kubota S, Oyama T

The plant circadian clock controls various physiological phenomena that are important for adaptation to natural day-night cycles. Many components of the circadian clock have been identified in Arabidopsis thaliana, the model plant for molecular genetic studies. Recent studies revealed evolutionary conservation of clock components in green plants. Homologues of clock-related genes have been isolated from Lemna gibba and Lemna aequinoctialis, and it has been demonstrated that these homologues function in the clock system in a manner similar to their functioning in Arabidopsis. While clock components are widely conserved, circadian phenomena display diversity even within the Lemna genus. In order to survey the full extent of diversity in circadian rhythms among duckweed plants, we characterised the circadian rhythms of duckweed by employing a semi-transient bioluminescent reporter system. Using a particle bombardment method, circadian bioluminescent reporters were introduced into nine strains representing five duckweed species: Spirodela polyrhiza, Landoltia punctata, Lemna gibba, L. aequinoctialis and Wolffia columbiana. We then monitored luciferase (luc+) reporter activities driven by AtCCA1, ZmUBQ1 or CaMV35S promoters under entrainment and free-running conditions. Under entrainment, AtCCA1::luc+ showed similar diurnal rhythms in all strains. This suggests that the mechanism of biological timing under day-night cycles is conserved throughout the evolution of duckweeds. Under free-running conditions, we observed circadian rhythms of AtCCA1::luc+, ZmUBQ1::luc+ and CaMV35S::luc+. These circadian rhythms showed diversity in period length and sustainability, suggesting that circadian clock mechanisms are somewhat diversified among duckweeds.

Ultraviolet radiation induces stress in etiolated Landoltia punctata, as evidenced by the presence of alanine, a universal stress signal: a 15N NMR study

Plant Biol. 2014 May 29. doi: 10.1111/plb.12198

Monselise EB, Levkovitz A, Kost D

Analysis with (15) N NMR revealed that alanine, a universal cellular stress signal, accumulates in etiolated duckweed plants exposed to 15-min pulsed UV light, but not in the absence of UV irradiation. The addition of 10 mm vitamin C, a radical scavenger, reduced alanine levels to zero, indicating the involvement of free radicals. Free D-alanine was detected in (15) N NMR analysis of the chiral amino acid content, using D-tartaric acid as solvent. The accumulation of D-alanine under stress conditions presents a new perspective on the biochemical processes taking place in prokaryote

and eukaryote cells.

Biochemical and standard toxic effects of acetaminophen on the macrophyte species Lemna minor and Lemna gibba

Environ Sci Pollut Res Int. 2014 Jun 3.

Nunes B, Pinto G, Martins L, Gonçalves F, Antunes SC

Acetaminophen is globally one of the most prescribed drugs due to its antipyretic and analgesic properties. However, it is highly toxic when the dosage surpasses the detoxification capability of an exposed organism, with involvement of an already described oxidative stress pathway. To address the issue of the ecotoxicity of acetaminophen, we performed acute exposures of two aquatic plant species, Lemna gibba and Lemna minor, to this compound. The selected biomarkers were number of fronds, biomass, chlorophyll content, lipid peroxidation (TBARS assay), and proline content. Our results showed marked differences between the two species. Acetaminophen caused a significant decrease in the number of fronds (EC50 = 446.6 mg/L), and the establishment of a dose-dependent peroxidative damage in L. minor, but not in L. gibba. No effects were reported in both species for the indicative parameters chlorophyll content and total biomass. However, the proline content in L. gibba was substantially reduced. The overall conclusions point to the occurrence of an oxidative stress scenario more prominent for L. minor. However, the mechanisms that allowed L. gibba to cope with acetaminophen exposure were distinct from those reported for L. minor, with the likely involvement of proline as antioxidant.

New flavanol and cycloartane glucosides from Landoltia punctata

Molecules 2014, 19: 6623-34.

Wang N, Xu G, Fang Y, Yang T, Zhao H, Li G

Chemical investigation on the constituents of Landoltia punctata led to the isolation and identification of 17 compounds, four of which were new and identified as (3b,24S)-9,19-cycloartane-3,22,24,25-tetraol 3-O-[b-D-glucopyranosyl-(1→2)]-[b-D-glucopyranosyl-(1→6)]-b-D-glucopyranoside (1), (3b,24S)-9,19-cycloartane-3,24,25-triol3-O-[b-d-glucopyranosyl(1→2)]-[b-D-glucopyranosyl-(1→6)]-b-D-glucopyranoside(2), 3,4'-dihydroxy-7,3'-dimethoxyflavan-5-O-b-D-glucopyranoside (3) and 3,4'-dihydroxy-4,7,3'-trimethoxyflavan-5-O-b-D-glucopyranoside (4). Their structures

were elucidated by spectroscopic, chemical, and biochemical methods. Thus, cycloartane triterpenoids were discovered in the Lemnaceae family for the first time. Compound 3 showed antioxidant capacity in the positively charged 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS+•) and superoxide anion radical scavenging assays.

Chromatin organisation in duckweed interphase nuclei in relation to the nuclear DNA content

Plant Biol. 2014 May 22. doi: 10.1111/plb.12194

Cao HX, Vu GT, Wang W, Messing J, Schubert I

The accessibility of DNA during fundamental processes, such as transcription, replication and DNA repair, is tightly modulated through a dynamic chromatin structure. Differences in large-scale chromatin structure at the microscopic level can be observed as euchromatic and heterochromatic domains in interphase nuclei. Here, key epigenetic marks, including histone H3 methylation and 5-methylcytosine (5-mC) as a DNA modification, were studied cytologically to describe the chromatin organisation of representative species of the five duckweed genera in the context of their nuclear DNA content, which ranged from 158 to 1881 Mbp. All studied duckweeds, including Spirodela polyrhiza with a genome size and repeat proportion similar to that of Arabidopsis thaliana, showed dispersed distribution of heterochromatin signatures (5mC, H3K9me2 and H3K27me1). This immunolabelling pattern resembles that of early developmental stages of Arabidopsis nuclei, with less pronounced heterochromatin chromocenters and heterochromatic marks weakly dispersed throughout the nucleus.

Assessing single and joint toxicity of three phenylurea herbicides using Lemna

minor and Vibrio fischeri bioassays

Chemosphere 2014 May 9. doi: 10.1016/j.chemosphere.2014.04.030

Gatidou G, Stasinakis AS, Iatrou EI

Single and joint toxicity of three substituted urea herbicides, namely monolinuron [3-(4-chlorophenyl)-1-methoxy-1-methylurea], linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and diuron [1-(3,4dichlorophenyl)-3,3 dimethyl urea], were studied. The duckweed Lemna minor

and the luminescent bacterium Vibrio fischeri were used for the toxicity assessment and they were exposed to various concentrations of the herbicides, individually and in binary mixtures. The exposure time was 7d for the duckweed and 30min for the bacterium. Estimation of EC50 values was performed by frond counting and reduction in light output for Lemna minor and Vibrio fischeri, respectively. Lemna minor was found to be much more sensitive than Vibrio fischeri to target compounds. The toxicity of the three herbicides applied solely was estimated to be in decreasing order: diuron (EC50=28.3μgL(-1))≈linuron (EC50=30.5μgL(-1))>monolinuron (EC50=300μgL(-1)) for the duckweed and linuron (EC50=8.2mgL(-1))>diuron (EC50=9.2mg L(-1))>monolinuron (EC50=11.2mgL(-1)) for the bacterium. Based on the environmental concentrations reported in the literature and EC50 values obtained from Lemna minor experiments, Risk Quotients (RQ) much higher than 1 were calculated for diuron and linuron. In Lemna minor experiments, combination of target compounds resulted to additive effects due to their same mode of phenylurea action on photosynthetic organisms. Regarding Vibrio fischeri, synergistic, additive and antagonistic effects were observed, which varied according to the concentrations of target compounds.

Relative in vitro growth rates of duckweeds (Lemnaceae) - the most rapidly growing higher plants

Plant Biol. 2014 May 6. doi: 10.1111/plb.12184

Ziegler P, Adelmann K, Zimmer S, Schmidt C, Appenroth K-J.

Relative growth rates (RGR), doubling times (DT) and relative weekly yields (RY) of 39 clones (ecotypes) from 13 species representing all five genera of duckweeds were determined under standardised cultivation conditions. RGR ranged overall from 0.153 to 0.519 day(-1) , DT from 1.34 to 4.54 days and RY from 2.9 to 37.8 week(-1) . The RGR and RY data can be compared directly to other published findings to only a limited extent on account of missing clonal designations for and limited accessibility to previously investigated clones, as well as the use of different data denominators. However, they are consistent with the published results of other comparative duckweed studies of similar scope in showing that RGR does not vary primarily at the level of the genus or species, but rather reflects the adaptation of individual clones to specific local conditions. The RGR data support the widely held assumption that

duckweeds can grow faster than other higher plants and that they can thus surpass land-based agricultural crops in productivity. Duckweeds are highly promising for the production of biomass for nutrition and energy, but extensive clonal comparison will be required to identify the most suitable isolates for this purpose.

Mallard duck (Anas platyrhynchos)-mediated dispersal of Lemnaceae: a contributing factor in the spread of invasive Lemna minuta?

Plant Biol. 2014 May 6. doi: 10.1111/plb.12182

Coughlan NE, Kelly TC, Jansen MA

Our ability to predict and manage the spread of alien, invasive plants is limited by a lack of understanding of dispersal potential. Invasive Lemna minuta has spread within a few decennia throughout Europe. However, the mechanism by which the species continues to spread remains a matter of speculation. In this study, hypothesised epizoochorous transport of L. minuta propagules by mallard ducks was investigated. Landolt (Biosystematic investigations in the family of duckweeds (Lemnaceae) (Vol. 2), The family of Lemnaceae - a monographic study (Vol. 1), 1986, Veröffentlichungen des Geobotanischen Institutes Der Eidg. Techniasche Hochschule, Stiftung Rübel, Zürich, Switzerland) referred to desiccation as the key limitation of the "colonization capability" of Lemnaceae. Therefore, we analysed retention of viability in L. minuta kept outside the liquid growth medium. Our data show prolonged viability of L. minuta fronds inserted between the feathers of a mallard duck. Consistently, the relative humidity between feathers ranged between 65% and 90%. Taking together evidence of entanglement and retention of L. minuta between the feathers of live ducks, with retention of viability, we consider it likely that mallards contribute to L. minuta dispersal. These data have implications for the management strategy of this invasive species.

cpDNA microsatellite markers for Lemna minor (Araceae): Phylogeographic implications

Appl Plant Sci. 2014 2(7) doi: 10.3732

Wani GA, Shah MA, Reshi ZA, Atangana AR, Khasa DP

A lack of genetic markers impedes our understanding of the population biology of Lemna minor. Thus, the development of appropriate genetic markers for L. minor promises to be highly useful for population genetic studies nd for addressing other life

history questions regarding the species. For the first time, we characterized nine polymorphic and 24 monomorphic chloroplast microsatellite markers in L. minor using DNA samples of 26 individuals sampled from five populations in Kashmir and of 17 individuals from three populations in Quebec. Initially, we designed 33 primer pairs, which were tested on genomic DNA from natural populations. Nine loci provided markers with two alleles. Based on genotyping of the chloroplast DNA fragments from 43 sampled individuals, we identified one haplotype in Quebec and 11 haplotypes in Kashmir, of which one occurs in 56% of the genotypes, one in 8%, and nine in 4%, respectively. There was a maximum of two alleles per locus. These new chloroplast microsatellite markers for L. minor and haplotype distribution patterns indicate a complex phylogeographic history that merits further investigation.

Phytoremediation Potential of Duckweed (Lemna minor L.) on Steel Wastewater

Int J Phytoremediation. 2014 Sep 5

Saha P, Banerjee A, Sarkar S

An eco-friendly and cost effective technique- phytoremediation was used to remediate contaminants from waste water. This study demonstrated that phytoremediation ability of duckweed (Lemna minor L.) to remove chloride, sulphate from Biological Oxygen Treatment (BOT) waste water of coke oven plant. The BOT water quality was assessed by analyzing physic-biochemical characters - pH, Biological oxygen demand (BOD), Chemical oxygen demand (COD), total dissolved solids (TDS) and elemental concentration. It was observed that an increase in pH value indicated an improvement of water quality. The experimental results showed that, duck weed effectively removed 30% chloride, 16% sulphate and 14% TDS from BOT waste water, which suggested its ability in phytoremediation for removal of chloride and sulphate from BOT waste water. A maximum increase of 30% relative growth rate of duck weed was achieved after 21 days of experiment. Thus, it was concluded that duckweed, an aquatic plant, can be considered for treatment of the effluent discharged from the coke oven plant.

Enhanced metabolic and redox activity of vascular aquatic plant Lemna valdiviana

under polarization in Direct Photosynthetic Plant Fuel Cell

Bioelectrochemistry. 2014 Aug 12. doi:10.1016

Hubenova Y, Mitov M

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±11mA/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.

Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species

Plant Biol 2014 Aug 12. doi: 10.1111/plb.12229

Borisjuk N, Chu P, Gutierrez R, Zhang H, Acosta K, Friesen N, Sree KS, Garcia C, Appenroth KJ, Lam E.

Lemnaceae, commonly called duckweeds, comprise a diverse group of floating aquatic plants that have previously been classified into 37 species based on morphological and physiological criteria. In addition to their unique evolutionary position among angiosperms and their applications in biomonitoring, the potential of duckweeds as a novel sustainable crop for fuel and feed has recently increased interest in the study of their biodiversity and systematics. However, due to their small size and abbreviated structure, accurate typing of duckweeds based on morphology can be challenging. In the past decade, attempts to employ molecular barcoding techniques for species assignment have produced promising results; however, they have yet to be codified into a simple and quantitative protocol. A study that compiles and compares the barcode sequences within all known species of this family would help to establish the fidelity and limits of this DNA-based approach. In this work, we compared the level of conservation between over 100 strains of duckweed for two intergenic barcode sequences derived from the plastid genome. By using over 300 sequences publicly available in the NCBI database, we determined the utility of each of these two barcodes for duckweed species identification. Through sequencing of these barcodes from additional accessions, 30 of the 37 known species of duckweed could be identified with varying levels of confidence using this approach. From our analyses using this reference dataset, we also confirmed two instances where mis-assignment of species has likely occurred. Potential strategies for further improving the scope of this

technology are discussed.

Arsenite Oxidation by the Phyllosphere Bacterial Community Associated with Wolffia australiana

Environ Sci Technol. 2014, 48:9668-74.

Xie WY, Su JQ, Zhu YG

Speciation is a key determinant in the toxicity, behavior, and fate of arsenic (As) in the environment. However, little is known about the transformation of As species mediated by floating macrophytes and the phyllosphere bacteria in aquatic and wetland environment. In this study, Wolffia australiana, a rootless floating duckweed, was cultured with (W+B) or without (W-B) phyllosphere bacteria to investigate its ability in arsenite (As(III)) oxidation. Results showed that sterile W. australiana did not oxidize As(III) in the growth medium or in plant tissue, whereas W. australiana with phyllpsphere bacteria displayed substantial As(III) oxidation in the medium. Quantitative PCR of As redox-related functional genes revealed the dominance of the arsenite oxidase (aioA) gene in the phyllosphere bacterial community. These results demonstrate that the phyllosphere bacteria were responsible for the As(III) oxidation in the W+B system. The rapid oxidation of As(III) by the phyllosphere bacterial community may suppress As accumulation in plant tissues under phosphate rich conditions. The aioA gene library showed that the majority of the phyllosphere arsenite-oxidizing bacteria related either closely to unidentified bacteria found in paddy environments or distantly to known arsenite-oxidizing bacteria. Our research suggests a previously overlooked diversity of arsenite-oxidizing bacteria in the phyllosphere of aquatic macrophytes which may have a substantial impact on As biogeochemistry in water environments, warranting further exploration.

Uranium and cadmium provoke different oxidative stress responses in Lemna minor L.

Plant Biol 2014 Jul 29. doi: 10.1111/plb.12222

Horemans N, Van Hees M, Van Hoeck A, Saenen E, De Meutter T, Nauts R, Blust R,

Vandenhove H

Common duckweed (Lemna minor L.) is ideally suited to test the impact of metals on freshwater vascular plants. Literature on cadmium (Cd) and uranium (U) oxidative

responses in L. minor are sparse or, for U, non-existent. It was hypothesised that both metals impose concentration-dependent oxidative stress and growth retardation on L. minor. Using a standardised 7-day growth inhibition test, the adverse impact of these metals on L. minor growth was confirmed, with EC50 values for Cd and U of 24.1 ± 2.8 and 29.5 ± 1.9 μm, respectively, and EC10 values of 1.5 ± 0.2 and 6.5 ± 0.9 μm, respectively. The metal-induced oxidative stress response was compared through assessing the activity of different antioxidative enzymes [catalase, glutathione reductase, superoxide dismutase (SOD), ascorbate peroxidase (APOD), guaiacol peroxidase (GPOD) and syringaldizyne peroxidase (SPOD)]. Significant changes in almost all antioxidative enzymes indicated their importance in counteracting the U- and Cd-imposed oxidative burden. However, some striking differences were also observed. For activity of APODs and SODs, a biphasic but opposite response at low Cd compared to U concentrations was found. In addition, Cd (0.5-20 μm) strongly enhanced plant GPOD activity, whereas U inhibited it. Finally, in contrast to Cd, U up to 10 μm increased the level of chlorophyll a and b and carotenoids. In conclusion, although U and Cd induce similar growth arrest in L. minor, the U-induced oxidative stress responses, studied here for the first time, differ greatly from those of Cd.

Effects of high ammonium level on biomass accumulation of common duckweed Lemna minor L.

Environ Sci Pollut Res Int. 2014 Jul 25

Wang W, Yang C, Tang X, Gu X, Zhu Q, Pan K, Hu Q, Ma D

Growing common duckweed Lemna minor L. in diluted livestock wastewater is an alternative option for pollutants removal and consequently the accumulated duckweed biomass can be used for bioenergy production. However, the biomass accumulation can be inhibited by high level of ammonium (NH4 (+)) in non-diluted livestock wastewater and the mechanism of ammonium inhibition is not fully understood. In this study, the effect of high concentration of NH4 (+) on L. minor biomass accumulation was investigated using NH4 (+) as sole source of nitrogen (N). NH4 (+)-induced toxicity symptoms were observed when L. minor was exposed to high concentrations of ammonium nitrogen (NH4 (+)-N) after a 7-day cultivation. L. minor exposed to the NH4 (+)-N concentration of 840 mg l(-1) exhibited reduced relative growth rate, contents of

carbon (C) and photosynthetic pigments, and C/N ratio. Ammonium irons were inhibitory to the synthesis of photosynthetic pigments and caused C/N imbalance in L. minor. These symptoms could further cause premature senescence of the fronds, and restrain their reproduction, growth and biomass accumulation. L. minor could grow at NH4 (+)-N concentrations of 7-84 mg l(-1) and the optimal NH4 (+)-N concentration was 28 mg l(-1).

Root length of aquatic plant, Lemna minor L., as an optimal toxicity endpoint for bio-monitoring of mining effluents

Integr Environ Assess Manag. 2014 Jul 7. doi: 10.1002/ieam.1558

Gopalapillai Y, Vigneault B, Hale BA

Lemna minor, a free-floating macrophyte, is used for bio-monitoring of mine effluent quality under the Metal Mining Effluent Regulations (MMER) of the Environmental Effects Monitoring (EEM) program in Canada, and is known to be sensitive to trace metals commonly discharged in mine effluents such as nickel. Environment Canada's standard toxicity testing protocol recommends frond count (FC) and dry weight (DW) as the two required toxicity endpoints - this is similar to other major protocols such as those by USEPA and OECD - which both require frond growth or biomass endpoints. However, we suggest that similar to terrestrial plants, average root length (RL) of aquatic plants will be an optimal and relevant endpoint. As expected, results demonstrate that RL is the ideal endpoint based on the three criteria: accuracy (i.e. toxicological sensitivity to contaminant), precision (i.e. lowest variance), and ecological relevance (metal mining effluents). Roots are known to play a major role in nutrient uptake in conditions of low nutrient conditions - thus having ecological relevance to freshwater from mining regions. Root length was the most sensitive and precise endpoint in this study where water chemistry varied greatly (pH and varying concentrations of Ca, Mg, Na, K, dissolved organic carbon and an anthropogenic organic contaminant, sodium isopropyl xanthates) to match mining effluent ranges. Although frond count was a close second, dry weight proved to be an unreliable endpoint. We conclude that the toxicity testing for the floating macrophyte should require average RL measurement as a primary endpoint.

Lead toxicity to Lemna minor predicted using a metal speciation chemistry approach

Environ Toxicol Chem. 2014 Jul 16. doi: 10.1002/etc.2688

Antunes PM, Kreager NJ

In the present study, predictive measures for Pb toxicity and Lemna minor were developed from bioassays with 7 surface waters having varied chemistries (0.5-12.5 mg/L dissolved organic carbon, pH of 5.4-8.3, and water hardness of 8-266 mg/L CaCO3). As expected based on water quality, 10%, 20%, and 50% inhibitory concentration (IC10, IC20, and IC50, respectively) values expressed as percent net root elongation (%NRE) varied widely (e.g., IC20s ranging from 306 nM to >6920 nM total dissolved Pb), with unbounded values limited by Pb solubility. In considering chemical speciation, %NRE variability was better explained when both Pb hydroxides and the free lead ion were defined as bioavailable (i.e., f{OH}) and colloidal Fe(III)(OH)3 precipitates were permitted to form and sorb metals (using FeOx as the binding phase). Although cause and effect could not be established because of covariance with alkalinity ($p = 0.08$), water hardness correlated strongly ($r(2) = 0.998$, $p < 0.0001$) with the concentration of total Pb in true solution ([Pb]T_True solution). Using these correlations as the basis for predictions (i.e., [Pb]T_True solution vs water hardness and %NRE vs f{OH}), IC20 and IC50 values produced were within a factor of 2.9 times and 2.2 times those measured, respectively. The results provide much needed effect data for L. minor and highlight the importance of chemical speciation in Pb-based risk assessments for aquatic macrophytes.

Manipulating duckweed through genome duplication

Plant Biol 2014, Jul 10. doi: 10.1111/plb.12212

Vunsh R, Heinig U, Malitsky S, Aharoni A, Avidov A, Lerner A, Edelman M

Significant inter- and intraspecific genetic variation exists in duckweed, thus the potential for genome plasticity and manipulation is high. Polyploidy is recognised as a major mechanism of adaptation and speciation in plants. We produced several genome-duplicated lines of Landoltia punctata (Spirodela oligorrhiza) from both whole plants and regenerating explants using a colchicine-based cocktail. These lines stably maintained an enlarged frond and root morphology. DNA ploidy levels determined by florescence-activated cell sorting indicated genome duplication. Line A4 was analysed after 75 biomass doublings. Frond area, fresh and dry weights, rhizoid number and length were significantly increased versus wild type, while the growth rate was

unchanged. This resulted in accumulation of biomass 17-20% faster in the A4 plants. We sought to determine if specific differences in gene products are found in the genome duplicated lines. Non-targeted ultra performance LC-quadrupole time of flight mass spectrometry was employed to compare some of the lines and the wild type to seek identification of up-regulated metabolites. We putatively identified differential metabolites in Line A65 as caffeoyl hexoses. The combination of directed genome duplication and metabolic profiling might offer a path for producing stable gene expression, leading to altered production of secondary metabolites.

Response of duckweed to various concentrations of selenite

Environ Sci Pollut Res Int. 2014 Jul 16.

Mechora S, Stibilj V, Germ M

The uptake of Se(IV) and its effects on the physiological and biochemical characteristics of duckweed (Lemna minor L.) have been studied. Duckweed plants were cultivated in controlled conditions for 7 weeks in different concentrations of Na selenite: 0.5, 1, 2, 5 (exposed 42 days) and 10 mg Se L(-1) (survived 7-21 days). The addition of 1 mg Se L(-1) did not negatively affect photochemical efficiency whilst respiratory potential increased in weeks 2-4 compared to control. The addition of 1 mg Se(IV) L(-1) increased the amount of chlorophyll a in weeks 3 and 4 and the amount of carotenoids in weeks 1, 3 and 5. Concentrations of 2 and 5 mg Se L(-1) negatively affected photochemical efficiency in weeks 3 and 4, and increased respiratory potential in comparison to the control in weeks 1-4, whilst beyond week 4, the respiratory potential decreased. Plants exposed to the highest concentration of Se(IV) had to be replaced twice during the experiment because they were dying. That was reflected in photochemical efficiency as well as in respiratory potential, which decreased in time. The content of Se in duckweed increased with the increasing concentration of Se: plants growing in 0.5 mg Se L(-1) contained 0.9 mg Se g(-1) DM and plants exposed to 5 mg Se L(-1) contained 5.8 mg Se g(-1) DM. The group of plants exposed to 10 mg Se L(-1) for 21 days contained 19.5 mg Se g(-1) DM. Our study revealed that duckweed absorbed high amount of Se(IV) from the water.

Toxicological effects of copper oxide nanoparticles on the growth rate, photosynthetic pigment content, and cell morphology of the duckweed Landoltia punctata

Protoplasma. 2014 Jul 9

Lalau CM, Mohedano RD, Schmidt EC, Bouzon ZL, Ouriques LC, Dos Santos RW, da Costa CH, Vicentini DS, Matias WG

Recently, the application of copper oxide nanoparticles (CuO-NPs) has increased considerably, primarily in scientific and industrial fields. However, studies to assess their health risks and environmental impacts are scarce. Therefore, the present study aims to evaluate the toxicological effects of CuO-NPs on the duckweed species Landoltia punctata, which was used as a test organism. To accomplish this, duckweed was grown under standard procedures according to ISO DIS 20079 and exposed to three different concentrations of CuO-NPs (0.1, 1.0, and 10.0 g L(-1)), with one control group (without CuO-NPs). The toxicological effects were measured based on growth rate inhibition, changes in the plant's morphology, effects on ultrastructure, and alterations in photosynthetic pigments. The morphological and ultrastructural effects were evaluated by electronic, scanning and light microscopic analysis, and CuO-NPs were characterized using transmission electron microscopy (TEM), zeta potential, and superficial area methods of analysis. This analysis was performed to evaluate nanoparticle size and form in solution and sample stability. The results showed that CuO-NPs affected morphology more significantly than growth rate. L. punctata also showed the ability to remove copper ions. However, for this plant to be representative within the trophic chain, the biomagnification of effects must be assessed.

Performance assessment of aquatic macrophytes for treatment of municipal wastewater

J Environ Health Sci Eng. 2014 Jul 16;12:106. doi: 10.1186/2052-336X-12-106.

Shah M, Hashmi HN, Ali A, Ghumman AR

The objective of the study was to evaluate the performance of three different aquatic macrophytes for treatment of municipal wastewater collected from Taxila (Pakistan). A physical model of treatment plant was constructed and was operated for six experimental runs with each species of macrophyte. Every experimental run consist of thirty days period. Regular monitoring of influent and effluent concentrations were

made during each experimental run. For the treatment locally available macrophyte species i.e. water hyacinth, duckweed & water lettuce were selected to use. To evaluate the treatment performance of each macrophyte, BOD5, COD, and Nutrients (Nitrogen and Phosphorus) were monitored in effluent from model at different detention time of every experimental run after ensuring steady state conditions. The average reduction of effluent value of each parameter using water hyacinth were 50.61% for BOD5, 46.38% for COD, 40.34% for Nitrogen and18.76% for Phosphorus. For duckweed the average removal efficiency for selected parameters were 33.43% for BOD5, 26.37% for COD, 17.59% for Nitrogen and 15.25% for Phosphorus and for Water Lettuce the average removal efficiency were 33.43% for BOD5, 26.37% for COD, 17.59% for Nitrogen and 15.25% for Phosphorus. The mechanisms of pollutant removal in this system include both aerobic and anaerobic microbiological conversions, sorption, sedimentation, volatilization and chemical transformations. The rapid growth of the biomass was measured within first ten days detention time. It was also observed that performance of macrophytes is influenced by variation of pH and Temperature. A pH of 6-9 and Temperature of 15-38°C is most favorable for treatment of wastewater by macrophytes. The option of macrophytes for treatment of Municipal sewage under local environmental conditions can be explored by further verifying the removal efficiency under variation of different environmental conditions. Also this is need of time that macrophyte system should be used for treatment of wastewater because their performance is comparable to conventional wastewater treatment plants and also the system has very low O&M costs.

Multispecies acute toxicity evaluation of wastewaters from different treatment stages in a coking wastewater-treatment plant

Environ Toxicol Chem. 2014, 33:1967-75.

Zhao JL, Jiang YX, Yan B, Wei C, Zhang LJ, Ying GG

Coking wastewater contributes approximately 5% of the total discharge volume of industrial wastewaters every year in China. The toxicity of coking wastewater to aquatic organisms is still unknown. The authors evaluated the toxicity of wastewater from different treatment stages in a coking wastewater treatment plant, South China, using 5 test species belonging to different trophic levels: luminous bacteria, green alga, a

crustacean, duckweed, and zebrafish embryos. The raw influent displayed the highest toxicity to the test species, with toxic units ranging from 16.2 to 1176. The toxicity in the wastewater was then gradually removed by sequential primary treatment, biological fluidized-bed treatment, and secondary clarifier treatment. The toxic unit of the final effluent was reduced to 2.26 for the green alga (Pseudokirchneriella subcapitata) and to 0 for the other 4 organisms. Quantitative analysis of metals and polycyclic aromatic hydrocarbons (PAHs) and qualitative scanning by gas chromatography-mass spectrometry showed the presence of a variety of pollutants in the coking wastewaters. Multivariate statistical analysis revealed that the toxicity in the coking wastewater was correlated to the chemical oxygen demand, total nitrogen, ammonia nitrogen, volatile phenols, sulfide, metals (Cr, As, Sb, Hg, Pb, and Ni), and ΣPAHs. Based on the results, it is required to set a safety emission limit value for the discharge of coking wastewater to protect aquatic organisms in the receiving water bodies.

Occurrence of pharmaceuticals in urban wastewater of north Indian cities and risk assessment.

Environ Monit Assess. 2014, 186: 6663-82.

Singh KP, Rai P, Singh AK, Verma P, Gupta S

Six pharmaceuticals of different categories, such as nonsteroidal anti-inflammatory drugs (ibuprofen, ketoprofen, naproxen, diclofenac), anti-epileptic (carbamazepine), and anti-microbial (trimethoprim), were investigated in wastewater of the urban areas of Ghaziabad and Lucknow, India. Samples were concentrated by solid phase extraction (SPE) and determined by high-performance liquid chromatography (HPLC) methods. The SPE-HPLC method was validated according to the International Conference on Harmonization guidelines. All the six drugs were detected in wastewater of Ghaziabad, whereas naproxen was not detected in Lucknow wastewater. Results suggest that levels of these detected drugs were relatively higher in Ghaziabad as compared to those in Lucknow, and diclofenac was the most frequently detected drug in both the study areas. Detection of these drugs in wastewater reflects the importance of wastewater inputs as a source of pharmaceuticals. In terms of the regional distribution of compounds in wastewater of two cities, higher spatial variations (coefficient of variation 112.90-459.44 %) were found in the Lucknow wastewater due to poor water exchange ability. In contrast, lower spatial variation (162.38-303.77 %) was observed in Ghaziabad. Statistical analysis results suggest that both data were highly skewed, and populations in two study areas were significantly different ($p < 0.05$). A risk assessment based on the calculated risk quotient (RQ) in six different bioassays (bacteria, duckweed, algae, daphnia, rotifers, and fish) showed that the nonsteroidal

anti-inflammatory drugs (NSAIDs) posed high (RQ >1) risk to all the test species. The present study would contribute to the formulation of guidelines for regulation of such emerging pharmaceutical contaminants in the environment.

Status of duckweed genomics and transcriptomics

Plant Biol. 2014 Jul 4. doi: 10.1111/plb.12201.

Wang W, Messing J

Duckweeds belong to the smallest flowering plants that undergo fast vegetative growth in an aquatic environment. They are commonly used in wastewater treatment and animal feed. Whereas duckweeds have been studied at the biochemical level, their reduced morphology and wide environmental adaption had not been subjected to molecular analysis until recently. Here, we review the progress that has been made in using a DNA barcode system and the sequences of chloroplast and mitochondrial genomes to identify duckweed species at the species or population level. We also review analysis of the nuclear genome sequence of Spirodela that provides new insights into fundamental biological questions. Indeed, reduced gene families and missing genes are consistent with its compact morphogenesis, aquatic floating and suppression of juvenile-to-adult transition. Furthermore, deep RNA sequencing of Spirodela at the onset of dormancy and Landoltia in exposure of nutrient deficiency illustrate the molecular network for environmental adaption and stress response, constituting major progress towards a post-genome sequencing phase, where further functional genomic details can be explored. Rapid advances in sequencing technologies could continue to promote a proliferation of genome sequences for additional ecotypes as well as for other duckweed species.

Efficient transformation and artificial miRNA gene silencing in Lemna minor

Plant Biol. 2014 Jul 2. doi: 10.1111/plb.12215.

Cantó-Pastor A, Mollá-Morales A, Ernst E, Dahl W, Zhai J, Yan Y, Meyers BC,

Shanklin J, Martienssen R

Despite rapid doubling time, simple architecture and ease of metabolic labelling, a lack of genetic tools in the Lemnaceae (duckweed) has impeded the full implementation of this organism as a model for biological research. Here, we present technologies to facilitate high-throughput genetic studies in duckweed. We developed a fast and

efficient method for producing Lemna minor stable transgenic fronds via Agrobacterium-mediated transformation and regeneration from tissue culture. Additionally, we engineered an artificial microRNA (amiRNA) gene silencing system. We identified a Lemna gibba endogenous miR166 precursor and used it as a backbone to produce amiRNAs. As a proof of concept we induced the silencing of CH42, a magnesium chelatase subunit, using our amiRNA platform. Expression of CH42 in transgenic L. minor fronds was significantly reduced, which resulted in reduction of chlorophyll pigmentation. The techniques presented here will enable tackling future challenges in the biology and biotechnology of Lemnaceae.

Growing duckweed for biofuel production: a review

Plant Biol. 2014 Jul 1. doi: 10.1111/plb.12216.

Cui W, Cheng JJ

Duckweed can be utilised to produce ethanol, butanol and biogas, which are promising alternative energy sources to minimise dependence on limited crude oil and natural gas. The advantages of this aquatic plant include high rate of nutrient (nitrogen and phosphorus) uptake, high biomass yield and great potential as an alternative feedstock for the production of fuel ethanol, butanol and biogas. The objective of this article is to review the published research on growing duckweed for the production of the biofuels, especially starch enrichment in duckweed plants. There are mainly two processes affecting the accumulation of starch in duckweed biomass: photosynthesis for starch generation and metabolism-related starch consumption. The cost of stimulating photosynthesis is relatively high based on current technologies. Considerable research efforts have been made to inhibit starch degradation. Future research need in this area includes duckweed selection, optimisation of duckweed biomass production, enhancement of starch accumulation in duckweeds and use of duckweeds for production of various biofuels.

Application of the name Lemna punctata G. Mey., the type of Landoltia Les & D. J. Crawford

Plant Biol. 2014 Jun 18. doi: 10.1111/plb.12209.

Wiersema JH

A recent (2011) attempt to change the previously designated type of the name of a duckweed species is discussed. Lemna punctata was first applied by Meyer in 1818 to a

plant collected from South America, but original specimens have not been located. A prior neotype designation associated this name with a species native to parts of Asia, Australia and the Pacific, and widely introduced elsewhere, including South America. The species is generally treated by taxonomists in the genus Spirodela (either as S. punctata or the synonym S. oligorrhiza) or, more recently, as the sole member of the new (1999) genus Landoltia (as L. punctata). If accepted, this 2011 attempt to re-neotypify L. punctata would disrupt the names of two duckweed species as well as that of Landoltia. Nomenclatural arguments against accepting this new typification are provided, therebysupporting the continued usage of Landoltia in the sense intended by its original authors.

Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production

Plant Biol. 2014 Jun 18. doi: 10.1111/plb.12204

Zhao Y, Fang Y, Jin Y, Huang J, Bao S, Fu T, He Z, Wang F, Wang M, Zhao H

The application potential of four duckweed strains from four genera, Wolffia globosa 0222, Lemna japonica 0223, Landoltia punctata 0224 and Spirodela polyrhiza 0225, were ompared in four parallel pilot-scale wastewater treatment systems for more than 1 year. The results indicated that each duckweed strain had unique potential advantages. Unlike L. japonica 0223 and La. punctata 0224, which grow throughout the year, S. polyrhiza 0225 and W. globosa 0222 do not survive cold weather. For year round performance, L. japonica 0223 was best not only in dry biomass production (6.10 g·m(-2) ·day(-1)), but also in crude protein (35.50%), total amino acid (26.83%) and phosphorus (1.38%) content, plus recovery rates of total nitrogen (TN), total phosphorus (TP) and CO2 (0.31, 0.085 and 7.76 g·m(-2) ·day(-1) , respectively) and removal rates of TN and TP (0.66 and 0.089 g·m(-2) ·day(-1) , respectively). This strongly demonstrates that L. japonica 0223 performed best in wastewater treatment and protein biomass production. Under nutrient starvation conditions, La. punctata 0224 had the highest starch content (45.84%), dry biomass production (4.81 g·m(-2) ·day(-1)) and starch accumulation (2.9 g·m(-2) ·day(-1)), making it best for starch biomass production. W. globosa 0222 and S. polyrhiza 0225 showed increased flavonoid biomass production, with higher total flavonoid content (5.85% and 4.22%, respectively) and high dominant flavonoids (>60%). This study provides useful information for selecting the appropriate local duckweed strains for further application in wastewater treatment and valuable biomass production.

Evaluation of phylogenetic relationships in Lemnaceae using nuclear ribosomal data

Plant Biol. 2014 Jun 18. doi: 10.1111/plb.12203

Tippery NP, Les DH, Crawford DJ

Nuclear DNA sequence data are essential for obtaining a complete understanding of plant species relationships, yet these data have been conspicuously absent from phylogenetic analyses of Lemnaceae (duckweeds). Using a modified Sanger sequencing protocol, we obtained DNA sequences of duckweed nuclear ribosomal regions, including 18S and 26S rDNA genes, the external transcribed spacer (ETS) and the frequently used internal transcribed spacer (ITS). After obtaining sequence data for all Lemnaceae species, we ascertained that prior difficulty in sequencing the ITS regions likely resulted from extremely rigid secondary structures, precipitated by a high proportion of G/C nucleotides. In phylogenetic analyses, nuclear ribosomal data largely supported relationships that had been inferred using chloroplast DNA sequence data.

Disposable electrochemical sensor to evaluate the phytoremediation of the aquatic plant Lemna minor L. toward Pb2+ and/or Cd2+

Environ Sci Technol. 2014, 48:7477-85.

Neagu D, Arduini F, Quintana JC, Di Cori P, Forni C, Moscone D

In this work a miniaturized and disposable electrochemical sensor was developed to evaluate the cadmium and lead ion phytoremediation potential by the floating aquatic macrophyte Lemna minor L. The sensor is based on a screen-printed electrode modified "in-situ" with bismuth film, which is more environmentally friendly than the mercury-based sensor usually adopted for lead and cadmium ion detection. The sensor was coupled with a portable potentiostat for the simultaneous measurement of cadmium and lead ions by stripping analysis. The optimized analytical system allows the simultaneous detection of both heavy metals at the ppb level (LOD equal to 0.3 and 2 ppb for lead and cadmium ions, respectively) with the advantage of using a miniaturized and cost-effective system. The sensor was then applied for the evaluation of Pb(2+) or/and Cd(2+) uptake by measuring the amount of the heavy metals both in growth medium and in plant tissues during 1 week experiments. In this way, the use of Lemna minor coupled with a portable electrochemical sensor allows the set up of a model system able both to remove the heavy metals and to measure "in-situ" the

magnitude of heavy metal removal.

Biochemical and standard toxic effects of acetaminophen on the macrophyte species Lemna minor and Lemna gibba

Environ Sci Pollut Res Int. 2014 Jun 3

Nunes B, Pinto G, Martins L, Gonçalves F, Antunes SC

Acetaminophen is globally one of the most prescribed drugs due to its antipyretic and analgesic properties. However, it is highly toxic when the dosage surpasses the detoxification capability of an exposed organism, with involvement of an already described oxidative stress pathway. To address the issue of the ecotoxicity of acetaminophen, we performed acute exposures of two aquatic plant species, Lemna gibba and Lemna minor, to this compound. The selected biomarkers were number of fronds, biomass, chlorophyll content, lipid peroxidation (TBARS assay), and proline content. Our results showed marked differences between the two species. Acetaminophen caused a significant decrease in the number of fronds (EC50 = 446.6 mg/L), and the establishment of a dose-dependent peroxidative damage in L. minor, but not in L. gibba. No effects were reported in both species for the indicative parameters chlorophyll content and total biomass. However, the proline content in L. gibba was substantially reduced. The overall conclusions point to the occurrence of an oxidative stress scenario more prominent for L. minor. However, the mechanisms that allowed L. gibba to cope with acetaminophen exposure were distinct from those reported for L. minor, with the likely involvement of proline as antioxidant.

Catalytic upgrading of duckweed biocrude in subcritical water

Bioresour Technol. 2014,166:37 - 44.

Zhang C, Duan P, Xu Y, Wang B, Wang F, Zhang L

Herein, a duckweed biocrude produced from the hydrothermal liquefaction of Lemna minor was treated in subcritical water with added H_2. Effects of several different commercially available materials such as Ru/C, Pd/C, Pt/C, Pt/γ-Al_2O_3, Pt/C-sulfide,

Rh/γ-Al_2O_3, activated carbon, MoS_2, Mo_2C, Co-Mo/γ-Al_2O_3, and zeolite on the yields of product fractions and the deoxygenation, denitrogenation, and desulfurization of biocrude at 350°C were examined, respectively. All the materials showed catalytic activity for deoxygenation and desulfurization of the biocrude and only Ru/C showed activity for denitrogenation. Of those catalysts examined, Pt/C showed the best performance for deoxygenation. Among all the upgraded oils, the oil produced with Ru/C shows the lowest sulfur, the highest hydrocarbon content (25.6%), the highest energy recovery (85.5%), and the highest higher heating value (42.6 MJ/kg). The gaseous products were mainly unreacted H_2, CH_4, CO_2, and C_2H6.

Comparison of biosorption and phytoremediation of cadmium and methyl parathion, a case-study with live Lemna gibba and Lemna gibba powder

Ecotoxicol Environ Saf. 2014, 105:112-20.

Halaimi FZ, Kellali Y, Couderchet M, Semsari S

Heavy metals and pesticides can be adsorbed by several biomasses such as living or non-living aquatic plants. In this study adsorption properties of live Lemna gibba and Lemna gibba powder were investigated with regard to cadmium and methyl parathion (MP). Toxicity data (IC50) on live L. gibba indicated that the period of four days was adequate for phytoremediation. Initial adsorption studies showed that both adsorbents were capable of removing cadmium and methyl parathion. Cadmium and methyl parathion adsorption onto L. gibba powder was fast and equilibrium was attained within 120min. The adsorption data could be well interpreted by the Freundlich model. The KF were: 7.8963 (Cd(2+)/ live Lemna); 0.7300 (MP/live Lemna); 11.5813 (Cd(2+)/Lemna powder); 1.1852 (MP/Lemna powder) indicating that Cd(2+) was more efficiently removed by both biosorbents than MP. Adsorption kinetics for cadmium and methyl parathion in both systems and rate constants were determined for each contaminant. It was found that the overall adsorption process was best described by pseudo-second-order kinetics. Boyd model and external mass-transfer expression were tested. It was concluded that cadmium and methyl parathion sorption onto Lemna powder is governed by film diffusion.

Rhizobium lemnae sp. nov., a bacterial endophyte of Lemna aequinoctialis

Int J Syst Evol Microbiol. 2014 Jul;64(Pt 7):2455-60. doi: 10.1099/ijs.0.061622-0

Kittiwongwattana C, Thawai C

Bacterial strain L6-16(T) was isolated from Lemna aequinoctialis. Cells were Gram-stain-negative, rod-shaped and motile with monopolar flagella. The phylogenetic analysis of its nearly complete 16S rRNA gene sequence revealed that strain L6-16(T) was a member of the genus Rhizobium. Its closest relative was Rhizobium tarimense PL-41(T) with a 16S rRNA gene sequence similarity value of 98.3%. Sequence similarity analysis of the housekeeping recA and atpD genes showed low levels of sequence similarity (<93.9%) between strain L6-16(T) and other species of the genus Rhizobium. Strain L6-16(T) was able to grow between pH 5 and 11 (optimum 7.0) and at temperatures ranging from 20 to 41 °C (optimum 30 °C). It tolerated NaCl up to 1 % (w/v) (optimum 0.5%). C18 : 1ω7c and/or C18 : 1ω6c (summed feature 8; 79.5%) were found as predominant cellular fatty acids. The DNA G+C content of strain L6-16(T) was 58.1 mol% (Tm). Based on low levels of DNA-DNA relatedness, strain L6-16(T) was distinct from members of phylogenetically related species including R. tarimense PL-41(T) (38.3 ± 0.8%), Rhizobium rosettiformans W3(T) (6.9 ± 0.4%) and Rhizobium pseudoryzae J3-A127(T) (12.3 ± 0.6 %). Strain L6-16(T) was unable to nodulate the roots of Phaseolus vulgaris, and nodC and nifH genes were not detected. The results obtained from phylogenetic analyses, phenotypic characterization and DNA-DNA hybridization indicated that strain L6-16(T) represents a novel species of the genus Rhizobium, for which the name Rhizobium lemnae sp. nov. is proposed. The type strain is L6-16(T) (= NBRC 109339(T)= BCC 55143(T)).

Sulfur metabolism: different tolerances of two aquatic macrophytes exposed to arsenic

Ecotoxicol Environ Saf. 2014,105:36-42.

Leão GA, Oliveira JA, Farnese FS, Gusman GS, Felipe RT

The toxicity of arsenic (As) and the mechanisms of response to this pollutant were analyzed in two aquatic plant species, one sensitive and one tolerant to the pollutant, Salvinia minima and Lemna gibba, respectively. The plants, grown in nutrient solution at pH 6.5, were exposed to As concentrations of 0.0 and 1.0mgL(-1) for 3 days. Both species accumulated As in their tissues, which resulted in increases in H2O2 production. L. gibba accumulated eleven times more As than S. minima. However, L. gibba was more tolerant, as shown by the absence of cell membrane damage and, despite greater accumulation, smaller growth reduction than S. minima. Indeed, the index of tolerance to As was twenty percent higher in L. gibba than in S. minima, which most likely results from the presence of a more efficient defense system. This defense system in L. gibba is most likely based on sulfate absorption, assimilation and metabolism. L. gibba showed an increase in sulfate absorption and adenosine-5'-triphosphate (ATP) sulfurylase activity (the first enzyme of the inorganic

sulfate assimilation pathway) following exposure to As. Consequently, the plant produced greater concentrations of sulfur-containing compounds that are involved in cellular detoxification, such as glutathione and non-protein thiols, and demonstrated greater enzymatic activity of γ-glutamylcysteine synthetase, glutathione S-transferase and glutathione reductase. Therefore, the plant's ability to increase absorption, assimilation and metabolism of sulfur are key steps for tolerance to oxidative stress triggered by metals.

Pyrolysis characteristics and kinetics of aquatic biomass using thermogravimetric analyzer

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The differences in pyrolysis process of three species of aquatic biomass (microalgae, macroalgae and duckweed) were investigated by thermogravimetric analysis (TGA). Three stages were observed during the pyrolysis process and the main decomposition stage could be divided further into three zones. The pyrolysis characteristics of various biomasses were different at each zone, which could be attributed to the differences in their components. A stepwise procedure based on iso-conversional and master-plots methods was used for the kinetic and mechanism analysis of the main decomposition stage. The calculation results based on the kinetic model was in good agreement with the experimental data of weight loss, and each biomass had an increasing activation energy of 118.35-156.13 kJ/mol, 171.85-186.46 kJ/mol and 258.51-268.71 kJ/mol in zone 1, 2 and 3, respectively. This study compares the pyrolysis behavior of various aquatic biomasses and provides basis for further applications of the biomass thermochemical conversion.

Genetic structure of duckweed population of Spirodela, Landoltia and Lemna from Lake Tai, China

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Duckweed is widely used in environmental biotechnology and has recently emerged as a potential feedstock for biofuels due to its high growth rate and starch content. The genetic diversity and composition of a natural duckweed population in genera Spirodela, Landoltia and Lemna from Lake Tai, China, were investigated using

probabilistic analysis of multilocus sequence typing (MLST). The 78 strains were categorized into five lineages, among which strains representing L. aequinoctialis and S. polyrhiza were predominant. Among the five lineages, interlineage transfers of markers were infrequent and no recombination was statistically detected. Tajima's D tests determined that all loci are subject to population bottlenecks, which is likely one of the main reasons for the low genetic diversity observed within the lineages. Interestingly, strains of L. turionifera are found to contain small admixture from L. minor, providing rare evidence of transfer of genetic materials in duckweed. This was discussed with respect to the hypothesis that a cross of these two gave rise to L. japonica. Moreover, the conventional maximum-likelihood phylogenetic analysis clearly recognized all the species in the three genera with high bootstrap supports. In conclusion, this work offers a basic framework for using MLST to characterize Spirodela, Landoltia and in particular Lemna strains at the species level, and to study population genetics and evolution history of natural duckweed populations.