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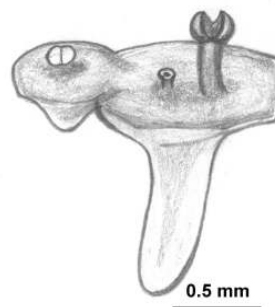
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Wolffia microscopica (Griff.) Kurz: Mother frond with budding daughter. This species was rediscovered in 2013 in India and Bangladesh. It is the fastest-growing Angiosperm known to date. Moreover, flowering is very frequent in this species. Note the mature anther lobes and stigma of the male and female floral organs arising out of the dorsally exposed floral cavity in the mother frond. Of special note is the attached daughter frond which is already flowering, showing the immature anther lobes. For more information about this fascinating duckweed species see Sree et al. (2014) *Flora* 210 (2015), 31-39. Drawing by Dr. K. Sowjanya Sree, India.



Letter from the Editor

Dear Duckweed Friends,

With this new issue of ISCDRA we want to wish you a Happy New Year 2015 – may it be a good year for you personally as well as for duckweed research and application. As you will see reading this issue, not only the year is new but also a lot within our ISCDRA issue 1/2015, number 7 is new as well. Some members of the committee discussed possible changes. One main point is that we want to publish the issues now regularly with 4 issues per year.

The drawing of one duckweed species was again prepared by K. Sowjanya Sree from Delhi, India. This time you can learn something more about *Wolffia microscopica*.

The year 2015 will be the year of the Third International Conference on Duckweed Research and Application. Our colleagues from Japan invite all interested people to participate this meeting from July 3-6 2015 in Kyoto. The homepage will be available soon at <http://www.duckweed2015.cosmos.bot.kyoto-u.ac.jp>. For requests, use shogoi@cosmos.bot.kyoto-u.ac.jp (Dr. Shogo Ito). Find more details within this issue.

Tamra Fakhoorian, member of ISCDRA, consulted on a duckweed mission in the Philippines. Read her colourful report in this issue. Eduardo Mercovich, also member of ISCDRA, reports about governmental support of a duckweed system in Argentina.

Marvin Edelman from Weizman Institute in Rehovot, Israel analysed all published papers about duckweed research concerning year of publication, topic and country. You can read his work "The State of Duckweed Affairs in the Literature" also in this issue.

"Plant Biology" published a special issue about the topic "*After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm?*" (eds. K.-J. Appenroth, D. J. Crawford. D. H. Les). This issue has been dedicated to the late Elias Landolt.

Also the chapter "From the Data Base" has now changed: subheadings might help you to find the articles you are interested in faster.

Best regards,

Klaus-J. Appenroth, Jena, 26th January 2015



The 3rd International Conference on Duckweed Research and Applications 2015 (ICDRA) and Call for Papers

The 3rd ICDRA will facilitate close interactions and coordination between duckweed researchers and application specialists from industries on a global scale. This workshop will be pivotal for charting a new course of development for this novel, micro-crop system by creating a new sense of teamwork and focus in the duckweed community. Working together, we can realize the remarkable potential of these plants in terms of biomass production and alleviation of urgent problems facing our planet today like waste water clean-up.

Organizers

- Chair: Tokitaka Oyama, Kyoto University
- Vice chair: Masaaki Morikawa, Hokkaido University
- Klaus-J. Appenroth, University of Jena, Germany
- Jay Cheng, North Carolina State University, USA
- Zhao Hai, Chengdu Institute of Biology, China
- Tamra Fakhoorian, International Lemna Association, USA
- Eduardo Mercovich, MamaGrande, Argentina

Venue: Science Seminar House of Faculty of Science, Kyoto University, Kyoto, JAPAN

Period: From 3rd to 6th July, 2015

Program (tentative)

- 3rd July: Registration and Reception
- 4th July: Scientific talks and Dinner
- 5th July: Scientific talks
- 6th July: Scientific talks, Excursion, and Closing ceremony

Call for Papers: for further inquiries- contact the Steering Committee at steering-committee@lemnedia.org and watch for updates on the conference website. All submissions will be reviewed and authors notified upon acceptance. For a listing of past conferences and presentations, visit http://lemnedia.org/wiki/International_Conference_of_Duckweed_Research_and_Applications

Registration fee: 40,000 yens (~116 yens/dollar, ~145 yens/euro, at Nov 17, 2014)

Housing: [Kyoto Garden Palace Hotel](#) (~8,000 yens / single night)



Duckweed Consulting Trip to the Philippines

By ILA Exec. Director Tamra Fakhoorian

Recently I traveled to Southeast Asia for eighteen days to consult on a duckweed pilot project headed by Mr. Tamerlane Sanchez, president of MP Wood Philippines Inc. Mangatarem, Pangasinan, Philippines.

MP Wood is focused on sustainable agriculture by supporting farmers with science, technology and training. MP Wood partners with municipalities, LGUs, NGOs and state universities & colleges to disseminate information to the public.

The MP Wood team is currently developing a lemna-based line of aquaculture, swine, and poultry feeds. My consulting objectives included development of procedures to decrease algal populations while increasing lemna production via organic fertilizers, exploration of the region for potential production sites and contribute expertise to the build-out of a solar dryer prototype.



Duckweed lab at Teraoka Farms



The Pangasinan region is known for its rice, mango, and more recently, bamboo production. The scenic tropical volcanic mountain range to the west offers late afternoon shade- perfect for the trial duckweed ponds at the company headquarters. The mountain range is also responsible for alkaline soil and water conditions. This created an ongoing challenge for duckweed to thrive over its primary competitor- filamentous algae.

I began my consulting work by conducting water quality tests and duckweed mat profiles. From there I did rough assays on several organic fertilizer sources from the region and developed test fertilizer regimes. The alkaline water issues were solved by water pretreatment, balancing existing nutrient loads and daily maintenance of the duckweed mats. I oversaw expansion of the pilot to include a novel biologically-self-sustaining duckweed production system.



Duckweed in hand



Solar dryer

Their solar dryer prototype was an adaptation of Paul Skillicorn's past work and my Kentucky model. I contributed additional details on exterior skin design, shelving access, chimney height, and solar tunnel design. As time ran short, I did not get to witness the first batch of solar dried duckweed in process. However, I can say that temperatures got so hot in the newly-built solar tunnel that the thermometer broke! (Estimated minimum of 65 degrees C.) This heated air is drawn into the drying chamber by a 1000 cfm heavy duty electric fan. Overall approximate size of solar dryer is 2 by 3.5 meters by 2 meters tall, with four easily accessible sliding trays. The solar tunnel is one meter wide at its base by 15 meters in length.

I was given a tour of a unique fish farm the last day of my stay. The concept was brilliant and forced me to throw out everything I knew about sustainable fish farming. MP Wood, Inc. and GET Farm of Rizal, Philippines have pioneered a "No-Discharge Tilapia Farming Method." Duckweed and rice bran are the primary feed ingredients.



GET owner Mang Erning, Tamra Fakhorian and MP Wood President, Tamerlane Sanchez



Tarlac College of Agriculture, in Tarlac, Philippines. From left to right, Consul Carlos Teraoka, President Dr. Max Guillermo, Tamra Fakhorian and Tamerlane Sanchez, President of MP Wood.



MP Wood President Sanchez has an agreement with Dr. Max P. Guillermo, president of Tarlac College of Agriculture, Philippines to do a more comprehensive study of the new system using the college's sixteen 50 x 50 meter ponds.

I would like to thank President Sanchez and the entire MP Wood team for their thoughtfulness and strong support of my work. They also saw to it that I was given the opportunity to experience everything from big city life in Manila to quiet village living. I was given tours of fish, watermelon, mango, mushroom, rice, and bamboo farms. I visited open air markets in several communities, hiked a few mountains and walked for miles beside picturesque rice paddies.



Fish farmer feeding duckweed to bangus for the first time.

I was introduced to dignitaries, educators, and students. I sampled many Filipino food delicacies and learned local customs. Most of all, I enjoyed the wit and warmth of each MP Wood team member and look forward to my return.

Baka pinagpala ang iyong trabaho. (May your work be blessed)

Inquiries should be directed to [Tamra Fakhoorian](mailto:tamraf9@gmail.com) Exec. Director ILA (tamraf9@gmail.com) or <http://www.internationallemnaassociation.org>



January 2015 Plant Biology Special Issue: Duckweed - Research and Application



Read Plant Biology's Special Issue 2015 dedicated to the late Prof. Dr. Elias Landolt (1926-2013) "After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm?" By K. -J. Appenroth, D. J. Crawford and D. H. Les, (pages 1–4).

Free access to the editorial letter is available at:

<http://onlinelibrary.wiley.com/doi/10.1111/plb.12248/pdf>

GUEST EDITORS:

- **K. -J. Appenroth**, Institute of Plant Physiology, Friedrich Schiller University, Dornburger Str. 159, 7743 Jena, Germany. E-mail: klaus.appenroth@uni-jena.de
- **D. J. Crawford**, Department of Ecology and Evolutionary Biology, and the Biodiversity Institute, University of Kansas, Lawrence, KS, USA.
- **D. H. Les**, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA.

Duckweeds (Lemnaceae), the fastest growing angiosperms, are increasingly gaining attention as potential crop plants for production of biomass. The availability of genome sequence data and effective techniques for genetic transformation in this plant family attracts researchers to reconsider their lost legacy as model plants. This issue presents interesting papers about duckweed systematics, physiology, molecular biology, biotechnology and heavy metal toxicity and is dedicated to late Elias Landolt (1926-2013), the "father" of modern duckweed research.

EDITORIAL Volume 17 (special issue), 2015

Supplementary material: Complete Bibliography of Elias Landolt (W. Laemmler).



ACUTE VIEW

Application of the name *Lemna punctata* G. Mey., the type of *Landoltia* Les & D. J. Crawford (pages 5–9). J. H. Wiersema

REVIEW ARTICLES

W. Wang and J. Messing. Status of duckweed genomics and transcriptomics (pages 10–15).

W. Cui and J. J. Cheng Growing duckweed for biofuel production: a review (pages 16–23)

U. Kutschera and K. J. Niklas. Darwin-Wallace Demons: survival of the fastest in populations of duckweeds and the evolutionary history of an enigmatic group of angiosperms (pages 24–32)

RESEARCH PAPERS

P. Ziegler, K. Adelman, S. Zimmer, C. Schmidt and K.-J. Appenroth. Relative *in vitro* growth rates of duckweeds (Lemnaceae) – the most rapidly growing higher plants (pages 33–41)

N. Borisjuk, P. Chu, R. Gutierrez, H. Zhang, K. Acosta, N. Friesen, K. S. Sree, C. Garcia, K. J. Appenroth and E. Lam. Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species (pages 42–49)

N. P. Tippery, D. H. Les and D. J. Crawford. Evaluation of phylogenetic relationships in Lemnaceae using nuclear ribosomal data (pages 50–58)

A. Cantó-Pastor, A. Mollá-Morales, E. Ernst, W. Dahl, J. Zhai, Y. Yan, B. C. Meyers, J. Shanklin and R. Martienssen. Efficient transformation and artificial miRNA gene silencing in *Lemna minor* (pages 59–65)

T. Muranaka, M. Okada, J. Yomo, S. Kubota and T. Oyama. Characterisation of circadian rhythms of various duckweeds (pages 66–74)

E. Lahive, J. O'Halloran and M. A. K. Jansen. A marriage of convenience; a simple food chain comprised of *Lemna minor* (L.) and *Gammarus pulex* (L.) to study the dietary transfer of zinc (pages 75–81)

Y. Zhao, Y. Fang, Y. Jin, J. Huang, S. Bao, T. Fu, Z. He, F. Wang, M. Wang and H. Zhao. Pilot-scale comparison of four duckweed strains from different genera for potential



application in nutrient recovery from wastewater and valuable biomass production (pages 82–90) N. Horemans, M. Van Hees, A. Van Hoeck, E. Saenen, T. De Meutter, R. Nauts, R. Blust and H.

N. Horemans, M. Van Hees, A. Van Hoeck, E. Saenen, T. De Meutter, R. Nauts, R. Blust and H. Vandenhove. Uranium and cadmium provoke different oxidative stress responses in *Lemna minor* L. (pages 91–100)

E. B.-I. Monselise, A. Levkovitz and D. Kost Ultraviolet radiation induces stress in etiolated *Landoltia punctata*, as evidenced by the presence of alanine, a universal stress signal: a ¹⁵N NMR study (pages 101–107)

N. E. Coughlan, T. C. Kelly and M. A. K. Jansen. Mallard duck (*Anas platyrhynchos*) -mediated dispersal of Lemnaceae: a contributing factor in the spread of invasive *Lemna minuta*? (pages 108–114)

SHORT RESEARCH PAPERS

R. Vunsh, U. Heinig, S. Malitsky, A. Aharoni, A. Avidov, A. Lerner and M. Edelman. Manipulating duckweed through genome duplication (pages 115–119)

H. X. Cao, G. T. H. Vu, W. Wang, J. Messing and I. Schubert. Chromatin organisation in duckweed interphase nuclei in relation to the nuclear DNA content (pages 120–124)

K.-J. Appenroth and L. Adamec. Specific turion yields of different clones of *Spirodela polyrhiza* depend on external phosphate thresholds (pages 125–129)

For some of these papers the abstract has been given already in ISCDRA Newsletter No. 6 (Early View).



Argentine Govt. supports Duckweed Integrated water treatment and Biomass Production Model

By Eduardo Mercovich, Co-founder, MamaGrande

A little more than a year ago, we imagined a scaleup project for MamaGrande (<http://MamaGrande.org>) based on our successful duckweed-based Totoras pilot wastewater to clean water + biomass full cycle process. It took one year to find the places, establish the necessary organizational architecture, partner bonding and agreements, write the proposal, and defend it against an international Jury, but eventually all those efforts bore fruit. Just before the end of 2014, the Argentinean Ministry of Science and Technology announced the winners and our project was the first.

What does this mean?

- For a couple million people in the NW of Argentina this means cleaner and more plentiful water for less price than before, just in time before a serious water crisis.
- For us, this means the opportunity to grow from a little less than 5 hectares to almost 150, cleaning water and harvesting huge amounts of rich biomass.
- For the Duckweed community all around the world, this means a real test of all these ideas we were working on for so long, and more opportunities for interaction, teaching and learning at the same time.
- For the rest of the world in warm to tropical climates, a blueprint of how they can treat various problems in an integrated, full cycle process, based on Mother Nature's laws. That is because we will not patent the process. Instead, we will publish it so anyone can use it.

The project is starting in February 2015, and this Government-backed phase will last for 3 years. Most of the money goes to plan & build a biorefinery that will convert starch into lactic acid to make a renewable & biodegradable plastic called PLA, and proteins into animal feed. More specific details are available in the Press Release #1, at http://mamagrande.org/pr/MamaGrande_PR_2014-11-20.pdf

We are very happy to share this huge step with everyone, and invite you all to participate, from our more applied folks at the ILA, to the more research-oriented ones in the ICDRA, and hope to see you in Tokyo 2015 where we will share and enjoy together.

Best wishes,

Eduardo Mercovich

Further inquiries may be directed to info@MamaGrande.org



The State of Duckweed Affairs in the Literature

By Marvin Edelman
Department of Plant and Environmental Sciences
Weizmann Institute of Science Rehovot, Israel

Duckweed, or Lemnaceae, is not just any field! It sports a two-volume 1,200 page bible: "*The family of Lemnaceae, Vol. 1 (morphology; karyology; ecology; geographic distribution; systematic position; nomenclature; descriptions) and Vol. 2 (phytochemistry; physiology; application; bibliography)*", compiled and written by Elias Landolt (with the inspired collaboration of Riklef Kandeler in Vol. 2) and published in 1986 and 1987 by the Swiss Federal Institute of Technology (ETH), Zurich. An excerpt from the introduction to Volume 1 is very informative. Landolt writes:

"Within the last sixty years the family of Lemnaceae became more and more important as suitable research material for plant physiology and phytochemistry. Some of the advantages of Lemnaceae are: fast and predominantly vegetative reproduction (genetically uniform clones), only small laboratory space requirement, and aseptical culture. In addition, the family aroused attention as a food source due to its high productivity and its high protein content. Furthermore, Lemnaceae are used as multipurpose test objects and for nutrient and mineral removal from sewage water."

Sound familiar? These are the same physiological and biotech points we are investigating and writing about today, 28 years later. However, not to worry; there is much new and exciting work going on as well. The substantial overlap is a result of how science is done. The generation-span of a scientific idea in the literature is likely to be from 15 to 20 years before it gets repeated by others: either knowingly, and then with refined technologies and deeper insight, or unknowingly, where more of the same leads to literature clutter.

Statistics are one means of revealing how a field is faring in the professional world. The exhaustive bibliography in Landolt's 1986-87 monograph contains more than 3,000 titles. What do the Lemnaceae literature statistics look like today? First of all, none of the top-of-the-line databases that I queried (PubMed, ISI Web of Science, Science Direct) came even close to matching Landolt's labor of love in completeness. ISI's Web of Science (using the 'All Databases' mode) was the closest, with 1,078 references for the period 1950-1986. The search terms I used were duckweed* OR Lemna OR Spirodela OR Wolffia OR Wolffia OR Landoltia.

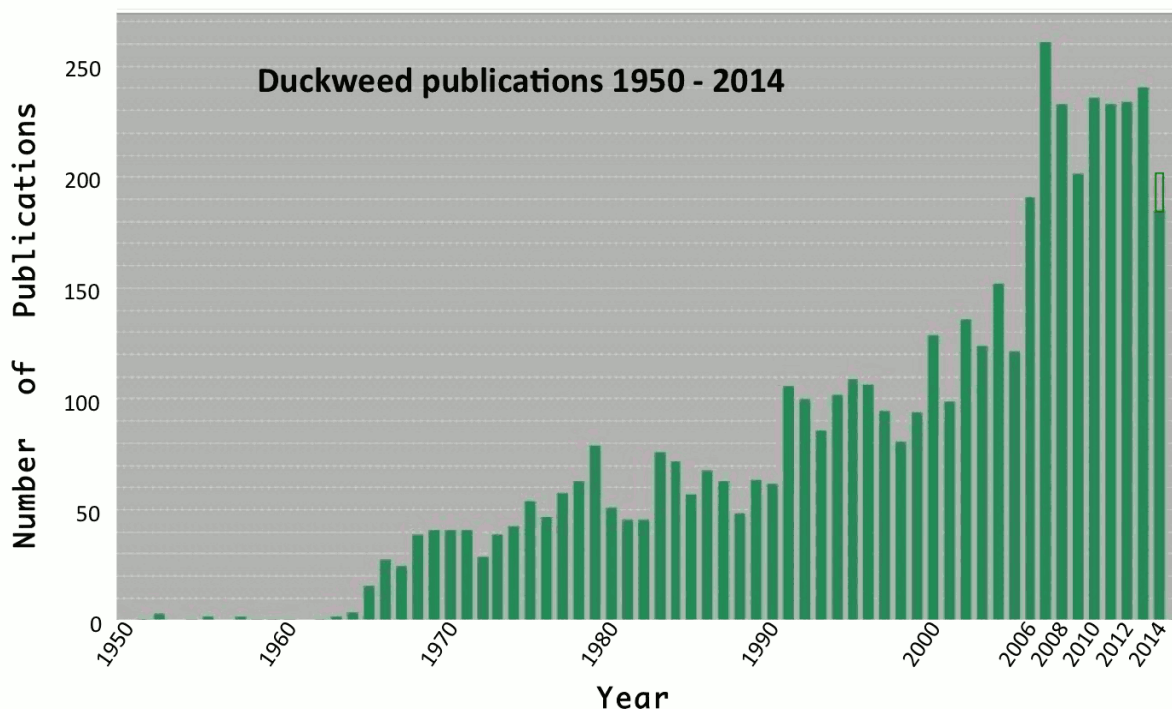
A scan of Landolt's reference list revealed that articles prior to 1950 represent a



surprisingly high 10% of the total. All told, ISI picked up about 40% of Landolt's exhaustive list. This is in line with the range of incompleteness that ISI achieved with my personal Lemnaceae reference list, where it lists just 25 of my 57 articles (44%). Looking deeper revealed that ISI and the other databases limit their search to Title, Abstract, and Keywords sections alone. If you don't toot your duckweed horn in these, you're out! On top of that, ISI missed some positives even in these sections and was lax in ferreting out book and symposia chapters.

Google!! If you want completeness, go to Google. However, there is a problem here too, but from the other end. Google Scholar (amid other search engines) is indeed complete, as any article worth its salt is referenced sometimes more than 100 times, thus rendering Google Scholar completely unsuitable for our purposes. The upshot of all this is that statistics extracted from the prevailing databases faithfully reveal trends as well as the big picture but need a fudge factor. In the case of ISI, of about *x2.5* (shown below in parentheses and italics) – to reach the theoretical 'Landolt level' of completeness.

Below is a snapshot of the ISI statistics, limited to articles, meetings, books, reviews and patents, but no abstracts.

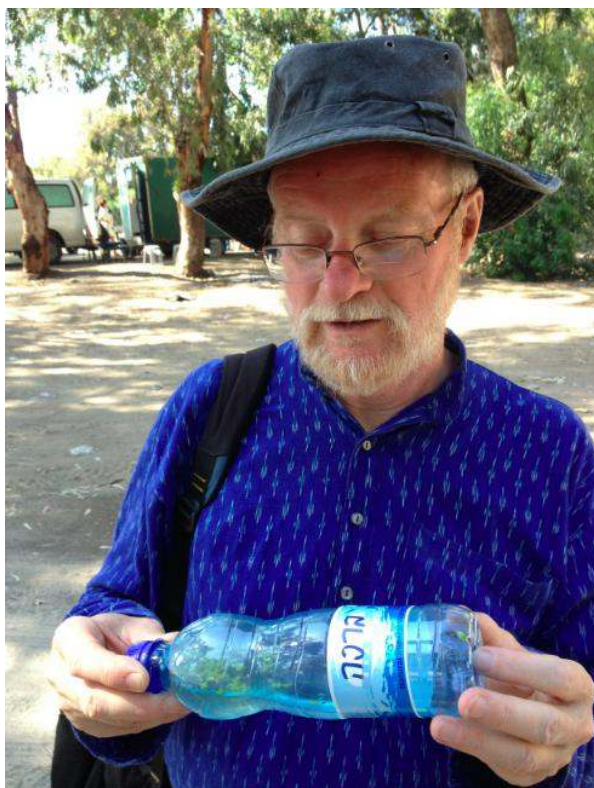


For the 65-year period of 1950-2014 (December 2014 not available as yet, estimate shown as green outline) the number of such publications came to 6,565 (*16,400*) and the



citations for these to 78,760 (195,000). There is a lag phase from 1950 to 1962, with 0 to 2 (5) publications appearing annually, followed by a rather leisurely linear rise in annual publication number to about 120 (300) by 2005. Then, in 2006, a duckweed publishing bloom materialized which has maintained itself ever since with an average of 220 (550) publications annually, such that 30% of all publications and 60% of all citations concerning duckweed have appeared since 2006.

Amazingly, publications from more than 100 countries are involved in these statistics, including my own country, Israel, where duckweed bloom annually.



Klaus Appenroth collecting duckweed samples from the same stream together with Sowjanya Sree and Marvin Edelman in May 2014.



The top ten countries in publication numbers are shown in the table below; to the left, for the entire 1950 - 2014 period, and to the right, for the 'bloom' period from 2006 - 2014.

1950-2014			2006-2014			
Rank	Country	Publications	Rank	Country	Publications	% recent
1	USA	790 (1975)	1	USA	259 (648)	22
2	Germany	341 (853)	2	China	188 (470)	82
3	Japan	339 (848)	3	India	149 (373)	57
4	India	262 (655)	4	Germany	146 (365)	43
5	Canada	254 (635)	5	Japan	98 (245)	29
6	China	229 (573)	6	Canada	93 (233)	37
7	Netherlands	175 (438)	7	Poland	80 (200)	47
8	Poland	172 (430)	8	Turkey	75 (188)	74
9	England	155 (388)	9	Portugal	46 (115)	68
10	France	139 (348)	10	Czech Rep.	46 (115)	74

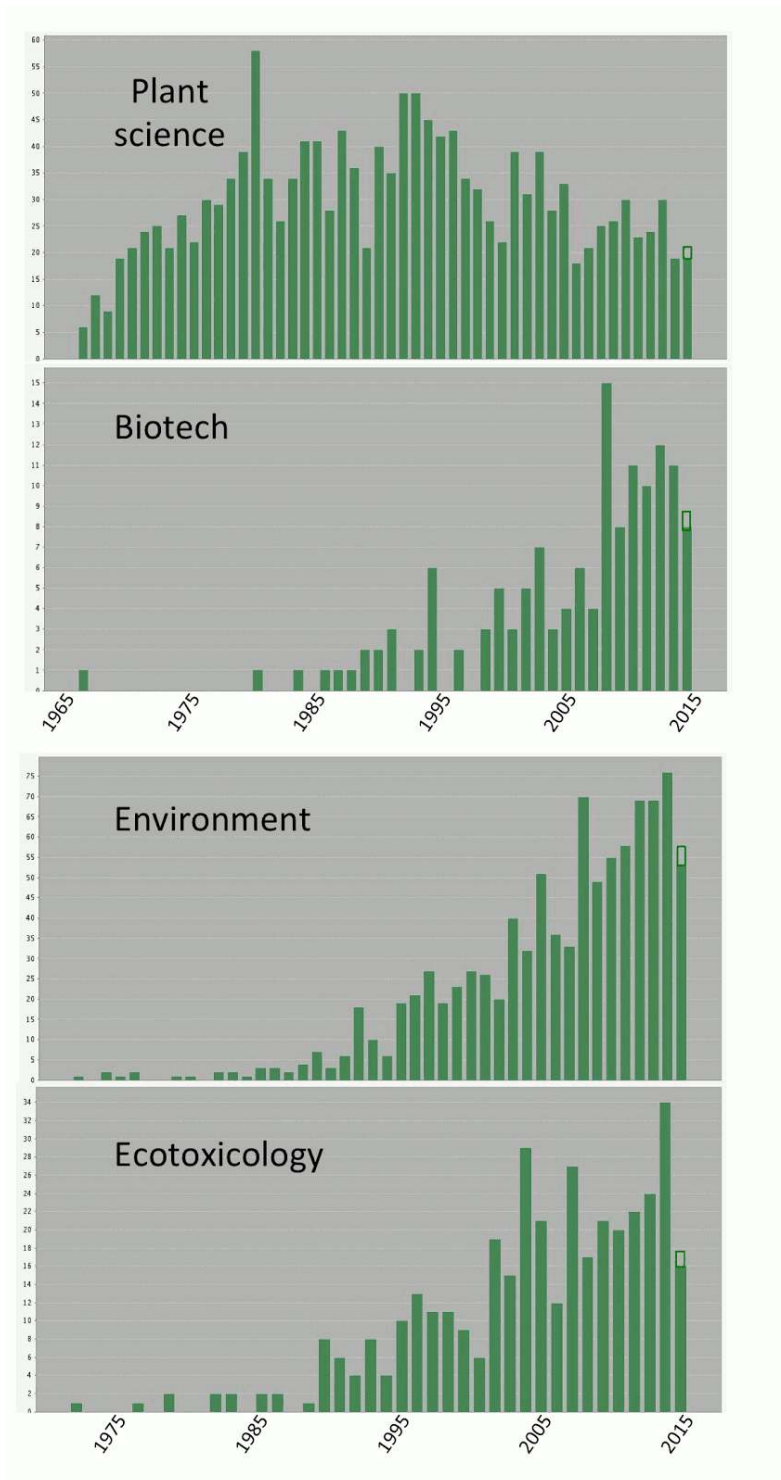
Three new countries made it to the top 10 for the period since 2006 (Turkey, Portugal, Czech republic) and a significant upgrade occurred for China and India. The last column shows the percent of total publications for each country in the recent bloom period. In half the cases, more than 50% of the total *Lemnaceae* literature output for that country had taken place. China is of special note. The emergence of China as a duckweed force and the rise in position of India in the literature heralds the change in research priorities that the duckweed community is currently experiencing.

What are these changes? The figure below makes its point as clear as duckweed purifies cedar swamps. Plant-science related publications, for fields such as biochemistry, molecular biology and physiology, the classical stalwarts of the duckweed literature, peaked in the '80s and '90s and has slowly receded since. However, at least one subfield, Biotechnology, is having its heyday. A significant portion of publications in this grouping relates to the fledgling field of duckweed transgenics and the increasing number of patents it is engendering.

Numerically, what has taken up the slack is the broad, growing interest in environmental aspects of duckweed research. The result: a pattern of publications relating to Environment that currently dominates the literature output for duckweed. The subfield of Ecotoxicology, with a pattern roughly mirroring its parent field, provides a significant



portion of the statistic.

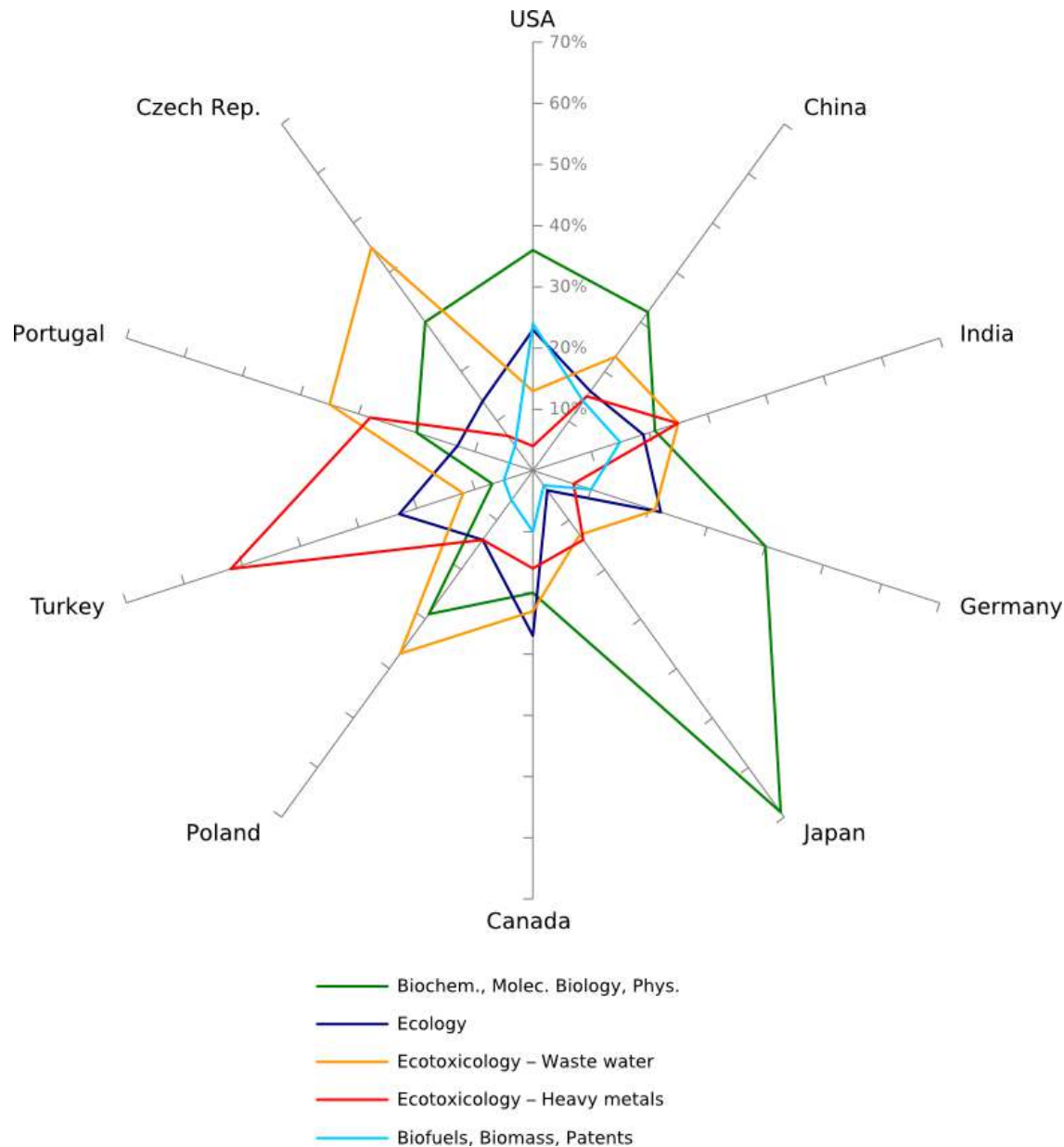


But who is doing what? The table below suggests that different countries and regions are



emphasizing different research interests and initiatives, undoubtedly driven by the aims of their respective funding agencies. Since the aggregate of the title, abstract and key words in many publications yields hits in multiple categories, I manually 'eye balled' all the titles in the "bloom period" (2006–2014) for the top 10 countries and subjectively decided which was the major category for each one. While low-tech and tedious, and cannot be done for large databases, the human factor does increase accuracy. (Consider for example, SwissProt vs. TREMBL) The duckweed literature was divided into three categories; 1. Plant Science, where the main issues are biochemistry, molecular biology and plant physiology, 2. Environment, which is further subdivided into Ecology and Ecotoxicology (separately categorized for Waste Water purification and Heavy Metal detoxification), and 3. Biotechnology, which bunches Biofuels, Biomass and duckweed Patents. For each country, the percentage of its publications falling in a particular category is tallied. The highest percentage category is shown in bold.

Area	Plant Science	Environment			Biotech
		Ecology	Ecotoxicology - Waste water	Ecotoxicology - Heavy metals	
Sub area	Biochem., Molec. Biology, Phys.				
2006 - 2014 Top 10	%	%	%	%	%
USA	36	23	13	4	24
China	32	16	23	15	14
India	21	19	25	25	15
Germany	40	22	21	7	10
Japan	69	4	13	14	3
Canada	20	27	23	16	10
Poland	29	14	37	14	6
Turkey	7	23	12	52	5
Portugal	20	13	35	28	4
Czech Rep.	30	14	45	7	5
<i>Averages</i>	30	18	25	18	9



The major category in recent USA duckweed publications remains in the basic sciences (36%). In addition, the USA leads in percentage of biotech publications (24%), with molecular genetic patents swelling the ranks. It is nice to learn that for China, too, the major publication category is the basic sciences (32%), with the remainder distributed rather evenly among the other categories and subcategories. This investment in basic research from an up-and-coming duckweed leader speaks well for the field. India appears to be concerned with more immediate issues in ecotoxicology, with an even distribution in the literature between waste water remediation (25%) and heavy metal detoxification (25%). Duckweed publications from Germany continue to show the



classical pattern with basic science papers (40%) in the lead. Contributing to this is the incisive series of publications on turion formation, physiology and biochemistry. Germany's high showing also in the ecology field (22%) goes well with the current European preoccupation with preserving the environment. Japan is heavily into basic duckweed research (69%), with the exciting rhizosphere studies contributing significantly to the total. Canada takes first place in being ecologically correct (27%), while Poland is putting its efforts into waste water cleanup (37%). Turkey on the other hand, is concentrating on heavy metal detoxification (52%), while Portugal and the Czech Republic join Poland in emphasizing waste water remediation (35% and 45%, respectively). Overall, Basic science publications represent 30% of publications from the Top 10 countries, while environmental studies (ecology and ecotoxicology) represent 61% and biotechnology, 9%. The mix has changed but variety remains and indicates a field in healthy development.

My take on where the duckweed literature will be by the end of the decade is colored by a recent signal event in the duckweed field: the publication of the complete genome of *Spirodela polyrhiza*. The molecular genetics possibilities that this now engenders are huge when coupled with the growing abilities by several groups to transform duckweed. Thus, I see a genome-driven increase in both basic research publications and biotechnology applications. Also, the intense interest in ecotoxicology is far from abating. All considered, my expectation is that we will continue to have a similar mix of duckweed literature as we have now, however with widened and deeper insight and much improved technology.



From the Data Base

Highlights

Duckweed in bloom: the 2nd International Conference on Duckweed Research and Applications heralds the return of a plant model for plant biology

Lam, Eric; Appenroth, Klaus J.; Michael, Todd; et al.

PLANT MOLECULAR BIOLOGY 84: 737-742 (2014)

More than 50 participants from around the world congregated at Rutgers University for 4 days to discuss the latest advances in duckweed research and applications. Among other developments in the field, exciting new information related to duckweed including genome sequencing, improved genetic transformation, and the identification of a novel plant growth promoting substance from bacteria were reported.

Biotechnology

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

Wang, Wenguo; Yang, Chuang; Tang, Xiaoyu; et al.

Environmental Science and Pollution Research International 21: 14202-10 (2014)

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 (NH_4^+) in non-diluted livestock wastewater and the mechanism of ammonium inhibition is not fully understood. In this study, the effect of high concentration of NH_4^+ on *L. minor* biomass accumulation was investigated using NH_4^+ as sole source of nitrogen (N). NH_4^+ -induced toxicity symptoms were observed when *L. minor* was exposed to high concentrations of ammonium nitrogen (NH_4^+ -N) after a 7-day cultivation. *L. minor* exposed to the NH_4^+ -N concentration of 840mg(-1) exhibited reduced relative growth rate, contents of carbon (C) and photosynthetic pigments, and C/N ratio. Ammonium ions 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 NH_4^+ -N concentrations of 7-84mg(-1) and the optimal NH_4^+ -N concentration was 28mg(-1).



Ecotoxicity of wood biomass leachates toward duckweed (*Lemna minor* L.)

Archanowicz, Ewa I.; Klosinska, Teresa; Antczak, Andrzej; et al.

PRZEMYSŁ CHEMICZNY 93: 1150-1154 (2014)

Poplar, oak, merbau, western redcedar, tatajuba and ipe wood biomass was leached with EtOH at 70 degrees C. The leachates were used for accelerating the duckweed growth and increasing the chlorophyll content at concns. 2.5, 10, 30 and 100 mg/dm³ in 96 h long study. The oak and ipe leachafes showed the highest toxicity against duckweed while the merbau leachate increased the biomass growth. The chlorophyll content decreased with increasing the concn. of the leachates.

Use of Duckweed (*Landoltia punctata*) as a Fermentation Substrate for the Production of Higher Alcohols as Biofuels

Su, Haifeng; Zhao, Yun; Jiang, Juan; et al.

ENERGY and FUELS 28: 3206-3216 (2014)

Duckweed (Lemnaceae) is a family of aquatic plants with potential for use as the next generation of alternative energy feedstocks, yet little related information about producing higher alcohols from duckweed has been published. We investigated the process of producing higher alcohols from duckweed via fermentation. Results showed that these plants have a promising future as the basis for developing biofuels. This could be achieved through fermentation by yeasts, producing not only traditional forms of energy such as ethanol but also higher alcohols with high energy yields through bioconversion by *Clostridium acetobutylicum*, mutant yeast strains, and bioengineered strains of *Escherichia coli*. The concentrations of butanol and total solvent produced via fermentation by *C. acetobutylicum* CICC 8012 were 12.03 and 20.03 g/L using acid hydrolysate of duckweed versus 12.33 and 20.05 g/L using enzymatic hydrolysate. The yields obtained of 24.06 g/L ethanol and 680.36 mg/L of isopentanol from duckweed using acid hydrolysate are 15 times higher than what could be obtained through the fermentation of the mutation of yeast. In addition, we were able to obtain yields of 16.27 mg/L butanol, 24.68 mg/L isopentanol, and 195.85 mg/L pentanol from the acid hydrolysate of duckweed via fermentation by the bioengineered strains of *E. coli*. Our results illustrated that duckweed represent an ideal fermentation substrate: they require only simple pretreatment, without the need for supplementary nitrogen or strengthening with redox agents. This provides a foundation for further development of industrialized biofuel production using duckweed.

Feed and Food



Concentration of metabolizable energy and digestibility of energy, phosphorus, and amino acids in *lemna* protein concentrate fed to growing pigs

Rojas, O. J.; Liu, Y.; Stein, H. H.

JOURNAL of ANIMAL SCIENCE 92: 5222-5229 (2014)

Lemna protein concentrate (LPC; 68.0% CP) is produced by extracting protein from de-oiled and dehydrated biomaterials from plants of the Lemnaceae family and may be used as a protein source for animals. There are, however, no published data on the nutritional value of LPC fed to pigs. Three experiments were, therefore, conducted to determine the concentration of ME, the standardized total tract digestibility (STTD) of P, and the standardized ileal digestibility (SID) of AA in LPC and to compare these values to values for fish meal and soybean meal (SBM). Experiment 1 was conducted to determine the ME of LPC, fish meal, SBM, and corn. Thirty-two barrows (initial BW: 16.8 +/- 2.8 kg) were placed in metabolism cages and allotted to a randomized complete block design with 4 diets and 8 replicate pigs per diet. A corn-based diet and 3 diets that contained corn and LPC, fish meal, or SBM were formulated. Feces and urine were collected for 5 d after a 5-d adaptation period, and all samples were analyzed for GE. Results indicated that the concentration of ME was not different among corn, fish meal, and SBM (3,855, 3,904, and 4,184 kcal/ kg DM, respectively), but there was a tendency ($P = 0.08$) for a reduced ME in LPC (3,804 kcal/kg DM) compared with SBM. In Exp. 2, 24 barrows (initial BW: 12.5 +/- 2.5 kg) were allotted to a randomized complete block design with 3 diets and 8 replicate pigs per diet and used to determine the STTD of P in LPC, fish meal, and SBM. Three diets that each contained 1 of the 3 test ingredients as the sole source of P were formulated. Pigs were placed in metabolism cages, and feces were collected for 5 d after a 5-d adaptation period. The STTD of P in LPC (72.8%) was not different from the STTD of P in fish meal (65.6%), but tended ($P = 0.07$) to be greater than in SBM (62.8%). The SID of AA in LPC, SBM, and fish meal was determined in Exp. 3. Eight barrows (initial BW: 21.4 +/- 4.0 kg) were equipped with a T-cannula in the distal ileum and randomly allotted to a replicated 4 x 4 Latin square design. A N-free diet and 3 cornstarch-based diets in which SBM, SBM and LPC or SBM and fish meal were the only sources of AA were formulated. The SID of most indispensable AA was greater ($P < 0.05$) in fish meal than in LPC, but the overall SID of AA was not different between fish meal and LPC. In conclusion, the ME and the STTD of P are not different between LPC and fish meal, but there is a tendency for greater ME in SBM than in LPC, whereas the STTD of P tends to be greater in LPC than in SBM. The SID of the most indispensable AA is greater in fish meal than in LPC.

Molecular Biology

Nitric oxide is involved in salicylic acid-induced flowering of *Lemna aequinoctialis* Welw.



Khurana, Ashima; Kumar, Rahul; Babbar, Shashi B.

ACTA PHYSIOLOGIAE PLANTARUM 36: 2827-2833 (2014)

Salicylic acid (SA) is a well-known inducer of flowering in *Lemna* under both non-inductive and inductive photoperiod conditions. However, the underlying mechanism is not well understood. Herein, we report for the first time that nitric oxide (NO) is partially involved in SA-induced flowering in *L. aequinoctialis* (Syn. *L. paucicostata* Hegelm.). Our results demonstrated that SA-induced flowering is significantly reduced by exogenous application of NO scavengers;

2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and methylene blue, nitric oxide synthase inhibitors; N-omega-nitro-l-arginine and N-omega-nitro-l-arginine-methyl ester hydrochloride, and nitrate reductase inhibitor; sodium tungstate in two strains of *Lemna* viz. 6746 and LP6. Altogether our present findings shed a light on the new role of NO in SA-induced flowering and open interesting directions that need further investigation.

Characterization of flower-inducing compound in *Lemna paucicostata* exposed to drought stress

Murata, Ariaki; Akaike, Ryota; Kawahashi, Tatsuya; et al.

TETRAHEDRON 70: 4969-4976 (2014)

A flower-inducing compound, LDS1, was isolated from a free-floating aquatic plant, *Lemna paucicostata*. The chemical structure and the absolute stereochemistry of LDS1 were determined as (9R,13R,11E,15Z)-9,13dihydroxy-10-oxooctadeca-11,15-dienoic acid for its most abundant diastereomer. LDS1 was enzymatically produced when the plant was exposed to drought stress, and induced flowering at a concentration of 10 nM.

Proteomic analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation

Huang, Mengjun; Fang, Yang; Xiao, Yao; et al.

INDUSTRIAL CROPS and PRODUCTS 59: 299-308 (2014)

Duckweed (*Landoltia punctata*) is widely distributed and has the capacity to remediate wastewater and produce tremendous biomass. Many studies have demonstrated that duckweed is a candidate feedstock for bioethanol production because of its relatively high starch and low lignin percentage. Nutrient starvation contributes to elevating starch content in duckweed, but the mechanism is so far unclear. In the research, the responses of *Landoltia punctata* to nutrient starvation were investigated using proteomics approach with iTRAQ-LC-MS/MS technology. A total of 2015 unique proteins were identified, with 172 proteins observed to be up-regulated and 43 down-regulated. Gene ontology (GO)



categorization analysis revealed that the biological process was significantly enriched (76.7%) in the metabolic process. Notably, in starch metabolism, the expression levels of enzymes involved in starch biosynthesis were up-regulated, whereas those involved in starch degradation showed no significant difference. The result was consistent with the transcriptomics data, enzymatic assay, and composition characterization. Importantly, in phenylpropanoid biosynthesis, several key enzymes involved in flavonoid biosynthesis showed up-regulated expression, but almost no enzyme related to the lignin biosynthetic branch exhibited sufficient expression abundance for detection. Proteomic analysis directly and powerfully demonstrated that high starch and low lignin percentage resulted from regulated expression of enzymes and alternation of metabolism flux in the relevant pathways. The proteomic results help us to understand the molecular mechanism of high starch accumulation and low lignin percentage, and promote the development of duckweed as a bioenergy crop.

Plant growth-promoting bacterium *Acinetobacter calcoaceticus* P23 increases the chlorophyll content of the monocot *Lemna minor* (duckweed) and the dicot *Lactuca sativa* (lettuce)

Suzuki, Wakako; Sugawara, Masayuki; Miwa, Kyoko; et al.

JOURNAL of BIOSCIENCE and BIOENGINEERING 118: 41-44 (2014)

Acinetobacter calcoaceticus P23 is a plant growth-promoting bacterium that was isolated from the surface of duckweed (*Lemna aoubilmsa*). The bacterium was observed to colonize on the plant surfaces and increase the chlorophyll content of not only the monocotyledon *Lemna minor* but also the dicotyledon *Lactuca sativa* in a hydroponic culture. This effect on the *Lactuca sativa* was significant in nutrient-poor (x 1/100 dilution of H2 medium) and not nutrient-rich (x 1 or x1/10 dilutions of H2 medium) conditions. Strain P23 has the potential to play a part in the future development of fertilizers and energy-saving hydroponic agricultural technologies.

Enhanced plant regeneration in *Lemna minor* by amino acids

Yang, Lin; Han, Huajun; Zuo, Zhaojiang; et al.

PAKISTAN JOURNAL of BOTANY 46: 939-943 (2014)

In present study we investigated the effects of different L-amino acids on the plant regeneration from callus of *Lemna minor*, and established an efficient protocol. Among the 20 L-amino acids, only L-Ser and L-Gly showed significant improving effect, with the optimal concentration being 1 mM and 1.5 mM, respectively. A regeneration frequency of 46% was observed when the callus transferred to the regeneration medium with addition of 1 mM L-Ser for 11 days. After 26 days of cultivation, the frond regeneration achieved 100% and 94% for 1 mM L-Ser and 1.5 mM L-Gly treatment, respectively.



Phytoremediation

BIOREMEDIATION OF AN IRON-RICH MINE EFFLUENT BY *LEMNA MINOR*

Teixeira, S.; Vieira, M. N.; Espinha Marques, J.; et al.

INTERNATIONAL JOURNAL of PHYTOREMEDIATION 16: 1228-1240 (2014)

Contamination of water resources by mine effluents is a serious environmental problem. In a old coal mine, in the north of Portugal (SAo Pedro da Cova, Gondomar), forty years after the activity has ended, a neutral mine drainage, rich in iron (FE) it stills being produced and it is continuously released in local streams (Ribeiro de Murta e Rio Ferreira) and in surrounding lands. The species *Lemna minor* has been shown to be a good model for ecotoxicological studies and it also has the capacity to bioaccumulate metals. The work aimed test the potential of the species *L. minor* to remediate this mine effluent, through the bioaccumulation of Fe, under greenhouse experiments and, at the same time, evaluate the time required to the maximum removal of Fe. The results have shown that *L. minor* was able to grow and develop in the Fe-rich effluent and bioaccumulating this element. Throughout the 21 days of testing it was found that there was a meaningful increase in the biomass of *L. minor* both in the contaminated and in the non-contaminated waters. It was also found that bioaccumulation of Fe (iron) occurred mainly during the first 7 days of testing. It was found that *L. minor* has potential for the bioremediation of effluents rich in iron.

Potential of *Lemna gibba* L. and *Lemna minor* L. for accumulation of Boron from secondary effluents

Tatar, Sule Yuksel; Obek, Erdal

ECOLOGICAL ENGINEERING 70: 332-336 (2014)

Aquatic plants are quite effective in removal of pollutants which cannot be removed in secondary treatment. *Lemna gibba* L. and *Lemna minor* L. among the aquatic plants were investigated for the removal of boron from the secondary waste water. Differences in Boron uptake were seen in the chosen aquatic macrophytes. Although boron is at low concentration in water, the bioaccumulated concentrations in *Lemna minor* L. and *Lemna gibba* L. were at high levels of between 140 mg Bg⁻¹ and 274 mg B g⁽⁻¹⁾, and 381 mg B g⁽⁻¹⁾ 523 mg B g⁽⁻¹⁾ respectively. From these results, it was understood that *Lemna gibba* L. is more prone to accumulate boron compared to *Lemna minor* L.

Adsorption Behavior of Methyl Violet 2B Using Duckweed: Equilibrium and Kinetics



Studies

Lim, Linda B. L.; Priyantha, Namal; Chan, Chin Mei; et al.

ARABIAN JOURNAL for SCIENCE and ENGINEERING 39: 6757-6765 (2014)

This study focuses on the adsorption behavior of using *Lemna minor* (Duckweed) as a potential low-cost biosorbent for the removal of methyl violet 2B (MV). The effects of parameters such as contact time (30-240 min), settling time (30-240 min), pH of dye solution from pH 2 to 12 were studied. Optimum contact time and settling time for the maximum removal of MV were 2 and 1 h, respectively. Maximum MV removal was observed at its ambient pH. Elemental analysis and functional group characterization of the biosorbent were carried out using X-ray fluorescence and Fourier-transformed infrared spectroscopy, respectively. Surface morphology of Duckweed, before and after adsorption of MV, was characterized using scanning electron microscope, and ionic strengths and pre-treatment of biosorbent were also investigated. Six different isotherm models, namely Langmuir, Freundlich, Temkin, Dubinin-Radushkevich, Redlich-Peterson and Sips isotherms, were performed on the equilibrium adsorption isotherm of MV. Experimental data for adsorption of MV correlate well with both the Langmuir and Sips isotherm models. The maximum biosorption capacity (q (max)) at 298 K was 332.5 mg g⁻¹ (Langmuir) and 307.3 mg g⁻¹ (Sips) for MV, which is far superior to most biosorbents reported. Kinetics study followed the pseudo-second order.

Sustainable Removal of Nitrophenols by Rhizoremediation Using Four Strains of Bacteria and Giant Duckweed (*Spirodela polyrhiza*)

Kristanti, Risky Ayu; Toyama, Tadashi; Hadibarata, Tony; et al.

WATER, AIR and SOIL POLLUTION 225: No 1928 (2014)

We examined the effectiveness of rhizoaugmentation for treating water contaminated with the nitrophenols (NPs), 2-NP, 3-NP, 4-NP, and 2,4-dinitrophenol (2,4-DNP) using NP-degrading bacteria. We used 2-NP-degrading *Pseudomonas* sp. (strain ONR1), 3-NP-degrading *Cupriavidus* sp. (MFR2), 4-NP-degrading *Rhodococcus* sp. (PKR1), 2,4-DNP-degrading *Rhodococcus* sp. (DNR2), and giant duckweed (*Spirodela polyrhiza*). The four bacterial strains readily colonized *Spirodela* roots, as approximately 1×10^5 colony-forming units [CFUs] plant⁻¹ to 10^6 - 10^7 CFU plant⁻¹. The higher populations remained stable through five sequential 2-day degradation cycles and completely removed all four NPs within each cycle. The root-bacteria association also successfully treated wastewater effluent contaminated with NPs; 52-71 % of 2-NP and 100 % of 3-NP, 4-NP, and 2,4-DNP were removed within each of five 2-day cycles. These results demonstrate the potential of rhizoaugmentation to achieve efficient and sustainable treatment of NP-contaminated waters.



Phytotoxicity

Arsenic uptake by *Lemna minor* in hydroponic system

Goswami, Chandrima; Majumder, Arunabha; Misra, Amal Kanti; et al.

INTERNATIONAL JOURNAL of PHYTOREMEDIATION 16 (12): 1221-1227 (2014)

Arsenic is hazardous and causes several ill effects on human beings. Phytoremediation is the use of aquatic plants for the removal of toxic pollutants from external media. In the present research work, the removal efficiency as well as the arsenic uptake capacity of duckweed *Lemna minor* has been studied. Arsenic concentration in water samples and plant biomass were determined by AAS. The relative growth factor of *Lemna minor* was determined. The duckweed had potential to remove as well as uptake arsenic from the aqueous medium. Maximum removal of more than 70% arsenic was achieved at initial concentration of 0.5mg/l arsenic on 15th day of experimental period of 22days. Removal percentage was found to decrease with the increase in initial concentration. From BCF value, *Lemna minor* was found to be a hyperaccumulator of arsenic at initial concentration of 0.5 mg/L, such that accumulation decreased with increase in initial arsenic concentration.

Assessing single and joint toxicity of three phenylurea herbicides using *Lemna minor* and *Vibrio fischeri* bioassays

Gatidou, Georgia; Stasinakis, Athanasios S; Iatrou, Evangelia I

Chemosphere 119: S69-S74 (2015)

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-(3,4 dichlorophenyl)-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=28.3mgL(-1))>linuron (EC50=30.5mgL(-1))>monolinuron (EC50=300mgL(-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

Cadmium removal by *Lemna minor* and *Spirodela polyrhiza*

Chaudhuri, Devaleena; Majumder, Arunabha; Misra, Amal K.; et al.

INTERNATIONAL JOURNAL of PHYTOREMEDIATION 16: 1119-1132 (2014)

The present study investigates the ability of two genus of duckweed (*Lemna minor* and *Spirodela polyrhiza*) to phytoremediate cadmium from aqueous solution. Duckweed was exposed to six different cadmium concentrations, such as, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0mg/L and the experiment was continued for 22days. Water samples were collected periodically for estimation of residual cadmium content in aqueous solution. At the end of treatment period plant samples were collected and accumulated cadmium content was measured. Cadmium toxicity was observed through relative growth factor and changes in chlorophyll content. Experimental results showed that *Lemna minor* and *Spirodela polyrhiza* were capable of removing 42-78% and 52-75% cadmium from media depending upon initial cadmium concentrations. Cadmium was removed following pseudo second order kinetic model. Maximum cadmium accumulation in *Lemna minor* was 4734.56mg/kg at 2mg/L initial cadmium concentration and 7711.00mg/kg in *Spirodela polyrhiza* at 3mg/L initial cadmium concentration at the end of treatment period. Conversely in both cases maximum bio-concentration factor obtained at lowest initial cadmium concentrations, i.e., 0.5mg/L, were 3295.61 and 4752.00 for *Lemna minor* and *Spirodela polyrhiza* respectively. The present study revealed that both *Lemna minor* and *Spirodela polyrhiza* was potential cadmium accumulator.

Adsorption of Cu (II), Mn (II) and Zn (II) by *Spirodela polyrhiza* (L.) Schleiden: Equilibrium, kinetic and thermodynamic studies

Meitei, Maibam Dhanaraj; Prasad, Majeti Narasimha Vara

ECOLOGICAL ENGINEERING 71: 308-317 (2014)

The potential of the free floating freshwater macrophyte *Spirodela polyrhiza* (L.) Schleiden from the phoomdi of Loktak lake, India as an adsorbent to remove Cu (II), Mn (II) and Zn (II) ions from single, binary and ternary metal solution system was investigated. Metal adsorption was fast and equilibrium was attained within 120 min. Langmuir isotherm best described the equilibrium with maximum adsorption capacities ($q(\max)$) of 52.6 mg g⁻¹ for Cu (II), 35.7 mg g⁻¹ for Mn (II) and 28.5 mg g⁻¹ for Zn (II) ions. Adsorption of the specific metal ions from binary and ternary metal solution system showed antagonistic nature due to screening effect and competition between the metal ions for active sites on



the biomass. The present study showed that *S. polyrhiza* biomass could be used as an effective, low-cost adsorbent for Cu (II), Mn (II) and Zn (II) ions removal from wastewaters. (C) 2014 Elsevier B.V. All rights reserved.

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

Antunes, Paula M. C.; Kreager, Nancy J.

ENVIRONMENTAL TOXICOLOGY and CHEMISTRY 33: 2225-2233 (2014)

In the present study, predictive measures for Pb toxicity and *Lemna minor* were developed from bioassays with 7 surface waters having varied chemistries (0.5-12.5mg/L dissolved organic carbon, pH of 5.4-8.3, and water hardness of 8-266mg/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., $f(\text{OH})$) 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 ($[\text{Pb}](T_{\text{True solution}})$). Using these correlations as the basis for predictions (i.e., $[\text{Pb}](T_{\text{True solution}})$ vs water hardness and %NRE vs $f(\text{OH})$), 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.

Effects of laser ablated silver nanoparticles on *Lemna minor*

Ucuncu, Esra; Ozkan, Alper D.; Kursungoz, Canan; et al.

CHEMOSPHERE 108: 251-257 (2014)

The present study investigates and models the effect of laser ablated silver nanoparticles (AgNPs) on the development of the aquatic macrophyte *Lemna minor*. Toxic effects of five different AgNP concentrations (8, 16, 32, 96 and 128 $\mu\text{g L}^{-1}$) on *L. minor* were recorded over seven days under simulated natural conditions. Biosorption of AgNPs by *L. minor* was modeled using four sorption isotherms, and the sorption behavior was found to agree most closely with the Langmuir-Freundlich model ($R^2 = 0.997$). While toxic effects of AgNPs could be observed in all models and concentrations, the greatest increase in toxicity was in the 8-32 $\mu\text{g L}^{-1}$ range. Dry weight- and frond number-based inhibition experiments suggest that growth inhibition does not necessarily scale with



AgNP concentration, and that slight fluctuations in inhibition rates exist over certain concentration ranges. Very close fits ($R^2 = 0.999$) were obtained for all removal models, suggesting that the fluctuations are not caused by experimental variation. In addition, *L. minor* was found to be a successful bioremediation agent for AgNPs, and displayed higher removal rates for increasing AgNP doses. FT-IR spectroscopy suggests that carbonyl groups are involved in AgNP remediation.

Effect of soluble copper released from copper oxide nanoparticles solubilisation on growth and photosynthetic processes of *Lemna gibba* L.

Perreault, Francois; Samadani, Mahshid; Dewez, David

NANOTOXICOLOGY 8: 374-382 (2014)

Copper oxide nanoparticles (CuO NPs) are used as a biocide in paints, textiles and plastics. Their application may lead to the contamination of aquatic ecosystems, where potential environmental effects remain to be determined. Toxic effects may be related to interactions of NPs with cellular systems or to particles' solubilisation releasing metal ions. In this report, we evaluated CuO NPs and soluble copper effects on photosynthesis of the aquatic macrophyte *Lemna gibba* L to determine the role of particle solubility in NPs toxicity. When *L. gibba* plants were exposed 48 h to CuO NPs or soluble copper, inhibition of photosynthetic activity was found, indicated by the inactivation of Photosystem II reaction centers, a decrease in electron transport and an increase of thermal energy dissipation. Toxicity of CuO NPs was mainly driven by copper ions released from particles. However, the bioaccumulation of CuO NPs in plant was shown, indicating the need to evaluate organisms of higher trophic level.

Others

Short-duration exposure to radiofrequency electromagnetic radiation alters the chlorophyll fluorescence of duckweeds (*Lemna minor*).

Senavirathna, Mudalige Don Hiranya Jayasanka; Takashi, Asaeda; Kimura, Yuichi

Electromagnetic Biology and Medicine 33: 327-34 (2014)

Plants growing in natural environments are exposed to radiofrequency electromagnetic radiation (EMR) emitted by various communication network base stations. The environmental concentration of this radiation is increasing rapidly with the congested deployment of base stations. Although numerous scientific studies have been conducted to investigate the effects of EMR on the physiology of humans and animals, there have been few attempts to investigate the effects of EMR on plants. In this study, we



attempted to evaluate the effects of EMR on photosynthesis by investigating the chlorophyll fluorescence (ChF) parameters of duckweed fronds. During the experiment, the fronds were tested with 2, 2.5, 3.5, 5.5 and 8GHz EMR frequencies, which are not widely studied even though there is a potentially large concentration of these frequencies in the environment. The duckweed fronds were exposed to EMR for 30min, 1h and 24h durations with electric field strength of 45-50V/m for each frequency. The results indicated that exposure to EMR causes a change in the non-photochemical quenching of the duckweeds. The changes varied with the frequency of the EMR and were time-varying within a particular frequency. The temperature remained unchanged in the duckweed fronds upon exposure to EMR, which confirms that the effect is non-thermal.

Reproducibility of effects of homeopathically potentised gibberellic acid on the growth of *Lemna gibba* L. in a randomised and blinded bioassay

Majewsky, Vera; Scherr, Claudia; Arlt, Sebastian Patrick; et al.

HOMEOPATHY 103: 113-126 (2014)

Reproducibility of basic research investigations in homeopathy is challenging. This study investigated if formerly observed effects of homeopathically potentised gibberellic acid (GA(3)) on growth of duckweed (*Lemna gibba* L.) were reproducible.

Duckweed was grown in potencies (14x-30x) of GA(3) and one time succussed and unsuccussed water controls. Outcome parameter area-related growth rate was determined by a computerised image analysis system. Three series including five independent blinded and randomised potency experiments (PE) each were carried out. System stability was controlled by three series of five systematic negative control (SNC) experiments. Gibbosity (a specific growth state of *L. gibba*) was investigated as possibly essential factor for reactivity of *L. gibba* towards potentised GA(3) in one series of potency and SNC experiments, respectively.

Only in the third series with gibbous *L. gibba* L. we observed a significant effect ($p=0.009$, F-test) of the homeopathic treatment. However, growth rate increased in contrast to the former study, and most biologically active potency levels differed. Variability in PE was lower than in SNC experiments. The stability of the experimental system was verified by the SNC experiments.

Gibbosity seems to be a necessary condition for reactivity of *L. gibba* to potentised GA(3). Further still unknown conditions seem to govern effect direction and the pattern of active and inactive potency levels. When designing new reproducibility studies, the physiological state of the test organism must be considered. Variability might be an interesting parameter to investigate effects of homeopathic remedies in basic research.



Links for Further Reading

<http://hp-test2-nacos.sakura.ne.jp/dw2015/#> Duckweed 2015 Conference site- watch for details as Kyoto, Japan conference preparations develop.

<http://duckweed2013.rutgers.edu/> Past duckweed conference papers and proceedings held at Rutgers University, New Brunswick, NJ in Aug, 2013

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

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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