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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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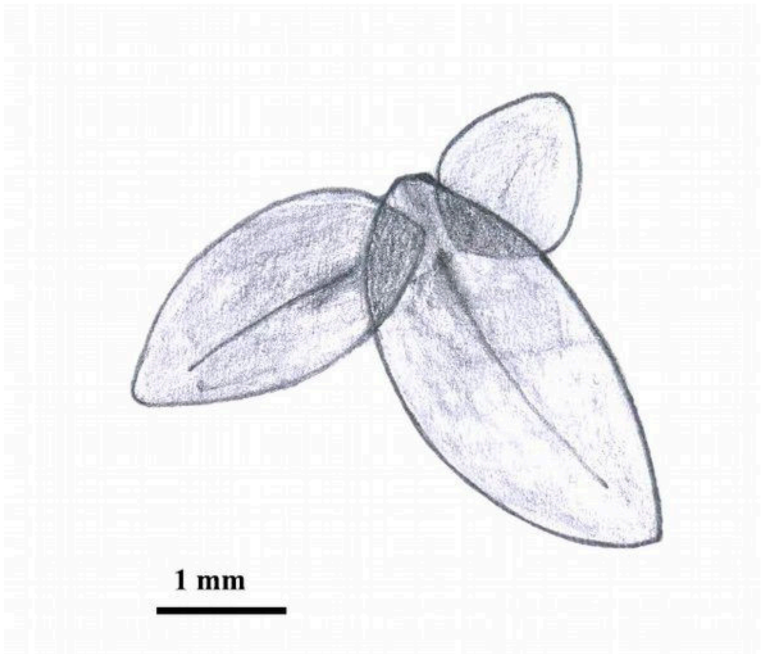
Lemna trisulca is a morphologically distinctive species within the genus *Lemna* with several fronds often cohere together, forming branched chains. This species is widely distributed in cooler climatic zones, such as Alaska and Siberia, of the world and local populations have been found in all inhabited continents except for South America. ***Wolffiella gladiata***, here shown as a colony of 3 fronds cohered together, is endemic in the southern regions of North America. ***Lemna turionifera*** can be found in North America in the upper latitudes and cooler regions of Europe and Asia. Fronds often have red pigmentation on the lower surface while a bit shiny on the upper side. Olive to brown colored turions, pictured here to the left from the cluster of fronds, can often be seen at end of the season and sink to the bottom of the water body. ***Lemna japonica*** is endemic to the eastern region of Asia. This species may have arose from the more wide-spread *Lemna minor*, as suggested by Landolt (1986) based on morphological considerations. While the close genetic relationship between *L. minor* and *L. japonica* is confirmed by more recent molecular studies using DNA barcodes (Borisjuk et al. 2015), it could also be consistent with a model in which these two species are sister taxa (Appenroth, unpublished). Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

In this issue

International Steering Committee on Duckweed Research and Applications Members

Information about the ISCDRA: http://lemnapedia.org/wiki/ISCDRA
All prior Duckweed Forum issues: http://lemnapedia.org/wiki/Duckweed_Forum

Science meets art: *Lemna valdiviana*



Lemna valdiviana Phil. is restricted to subtropical and tropical areas of both North and South America. Its presence is well reported in USA and Argentina. It is often confused with other *Lemna* species, e.g. *L. minuta*. However, its delineation from *L. yungensis* is not clear, as neither morphological nor genetic markers show unambiguous differences between the two *Lemna* species. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the editor

Dear friends of duckweed research and applications,

The **4th International Conference on Duckweed Research and Applications** (ICDRA-2017), which will be held at the Central University of Kerala in Periye, Kerala, India, from 23rd to 26th of October 2017, is fast approaching. We, together with Dr. K. Sowjanya Sree (Chair of ICDRA-2017) and Prof. Dr. Jitendra P. Khurana (Co-Chair of ICDRA-2017) would like to invite you to participate in this conference. We also hope that this will be an opportunity for a majority constituents of the duckweed community to meet each other and to discuss the most urgent questions in duckweed research and applications. We are sure that you will also enjoy very much the environment of this conference's venue situated at the most southern state of India, Kerala. In this "Duckweed Forum", issue N° 17, you will find the informational brochure of ICDRA-2017 as well as the web link of the conference for registration and abstract submission processes that are now open. If you need any help, please do not hesitate to contact the chair of the conference, Dr. K. Sowjanya Sree, as per the contact details given in the brochure as well as the web page of ICDRA-2017.

Closely connected with our conference is the **election of new members** of the "International Steering Committee on Duckweed Research and Applications" (ISCDRA). You have still the opportunity to suggest candidates. The order for the election has been modified to give more people the opportunity to vote. After the close of the nomination of candidates (this will be 6 weeks before the beginning of the ICDRA-2017) we will be open for electronic voting. During the "General Assembly" towards the end of the ICDRA-17, the service will be passed from the present committee to the newly elected one.

In the main part of our "Duckweed Forum" Prof. Dr. Marcel Jansen and his coworkers from the Cork University College, Ireland report about their research on duckweeds being transported by several animals and discuss about the consequences of the outdoor mass cultivation of a introduced species of duckweed.

In the chapter "Student Spotlight" Ömer Faruk Coşkun from the Erciyes University in Turkey is introduced, and in "Useful Methods 7" you will get an update to cultivation, sterilization and stock cultivation of duckweeds by K. Sowjanya Sree and Klaus-J. Appenroth.

Of course, there are again photos of four duckweed species from the Rutgers Duckweed Stock Cooperative on the cover page made by Eric Lam, and K. Sowjanya Sree presents a drawing of *Lemna valdiviana* Phil. under "Science meets Art". And finally, as it is common, the newsletter is appended by the recent literature on Lemnaceae in the chapter "From the data base".

We would like to remind you of our "Discussion corner", which awaits your contribution about all possible duckweed-related topics. We are open to discuss about the experiences in fighting algae in duckweed cultures.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),

Klaus-J. Appenroth, Chair

4th International Conference on Duckweed Research and Applications

23-26 October, 2017

Dept. of Environmental Science, Central University of Kerala, India

ICDRA - 2017
23-26 October, CU Kerala, INDIA

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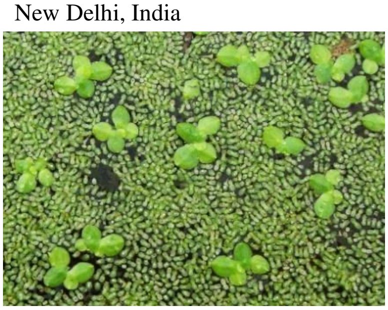
University of Delhi South Campus
New Delhi, India



About the conference

Duckweeds, small and fast growing aquatic plants belonging to Lemnaceae, are gaining attention both from basic research and application perspective. The first three conferences held biennially starting from 2011 in China, USA and Japan respectively have witnessed a growing interest of the scientific and industrial community in advancing the basic research on duckweeds together with realizing its numerous applications. We hope that this conference would provide a boost for the emerging duckweed research and also wish that it would foster the interactions between academia and industry.

Topics of the conference

- Duckweed: Basic biology
- Duckweed: Genetics and molecular biology
- Duckweed- Microbe interactions
- Practical applications of duckweeds

Registration

Registration category	Indian (INR)	Foreign (USD)
Academia (Research & Teaching)	9,000.00	400.00
Student	5,000.00	250.00
Non-academia/ Industry affiliated	15,000.00	600.00
Accompanying person	5,000.00	250.00

Registration fee is non-refundable.

Registration closes on 23rd August, 2017

Registration fee includes admission to all scientific sessions, conference kit, tea breaks, lunch and dinner on the conference days, transport to and from conference venue, entry to the cultural event.

Abstract submission deadline: 15th August, 2017

Please visit the web page for further information

http://icdra2017.cukerala.ac.in/Home.html

Venue: Central University of Kerala is located in Periye, Kasaragod (District) which is the northern most district of Kerala. Kerala is the southern most state of India and is popularly known as the God's own country for the natural beauty embedded in it. The University campus itself is located in a serene environment with the Western Ghats (one of the three biodiversity hotspots in the country) running parallel to the west coastline of India.

How to reach the venue?

By air: The closest airport is situated in Mangalore, Karnataka, India. The Bajpe International airport at Mangalore is having direct flights to Dubai, Abu Dhabi and Sharjah (connecting hubs). Mangalore is also well connected to other international airports in India: Mumbai, Bangalore, Hyderabad and Delhi. The ~80 kms stretch from airport to the venue, along the west coast, can be covered either by road (2.5 h) or by railway (1.5 h).

By road: Direct buses are available from different parts of Kerala. The University main gate is conveniently located on the National Highway with a halt for all local and express bus services passing enroute.

By train: The closest major railway station is situated 12 kms away at Kanhangad, which receives trains from various parts of the country. More information on train timing and availability may be obtained from www.indianrail.gov.in . Kanhangad is well connected by bus facility to the Central University campus at Periye.

Contact information

Dr. K. Sowjanya Sree
Dept. of Environmental Science
Central University of Kerala
River side Transit Campus
Padanakkad, Kasaragod
Kerala- 671314, India
Ph.: +91 9999672921
Email: ksowsree9@cukerala.ac.in; ksowsree@gmail.com

Announcing Call and rules for Nominees to the ISCDRA and election 2017

Order for the election of the members to the International Steering Committee on Duckweed Research and Applications.

The International Steering Committee on Duckweed Research and Applications (ISCDRA, http://lemnapedia.org/wiki/ISCDRA) was founded during the 2nd International Congress on Duckweed Research and Applications (ICDRA) held at Rutgers, the State University of New Jersey, New Brunswick, NJ in 2013.

Members of the committee cooperate with each other and the Duckweed Community in order to steer and coordinate activities of in the duckweed community to help promote academic research and commercial applications. Publishing the ISCDRA Newsletter "Duckweed Forum" is one of the obligations, among others.

1) The ISCDRA should consist of 5 members who will be elected before the biannual ICDRA in a secret electronic poll.

2) Anyone who has previously attended any of the ICDRA or will be attending it this year, or receives the ISCDRA Newsletter "Duckweed Forum" can suggest potential candidates including themselves up to 6 weeks before the meeting. For the election 2017 this will be 8th of September 2017 as the conference is from 23rd to 26th of October 2017 at the Central University of Kerala in Periye, District Kasaragod, Kerala, India (Chair Prof. Ass. Dr. K. Sowjanya Sree, email ksowsree9@cukerala.ac.in). Suggestions may be sent to the present head of the ISCDRA- Dr. Klaus J. Appenroth election@lemnapedia.org up to 6 weeks before the conference.

3) Voting will be done electronically, via email to the current ISCDRA designated members between 4 week and 2 week prior to the ICDRA conference. Deadline: 2nd of October 2017.

4) The right of voting will be granted to everyone who is an active member of our duckweed community. Beside people who attended one of the previous ICDRA or will attend the upcoming conference this year, the following people have the right of voting.

In Academia:

- A principle investigator who is working with duckweed in his/her laboratory.
- A researcher/postdoc/student who is working with duckweed in their research (i.e. not just reading about it, but actually doing a project).
- An administrator in a University or Funding Agency who has sufficient interest in the duckweed community to attend one of the previous or upcoming 4th ICDRA.

In Industry:

- An entrepreneur who is working to commercialize a duckweed-based technology and/or product.
- A research scientist who is working on a commercial venture that aims to develop a duckweed-based technology and/or product.
- A venture capital principle who has invested significantly into duckweed-based technology and/or product.

- A worker in a commercial venture involved with a duckweed-based technology and/or product who has attended one of the previous or upcoming 4th ICDRA.
- A contributing member of the International Lemna Association.

5) From the list of candidates, a maximum of 5 candidates could be voted for.

6) The five newly elected members will be notified by email and they will elect the head of the committee before or during the ICDRA.

7) In case that by chance all elected members are either from the applied field or from the research field, the elected Chair will appoint one additional member from the missing field.

8) During the General Assembly of the ISCDRA meeting, the previous Committee reports shortly about the activities since the previous election and the duty is transferred to the newly elected ISCDRA.

31st March 2017

Flying duck(weed)s

Marcel A.K. Jansen*, Neil Coughlan, Simona Paolacci, Ronan Bonfield, and Tom Kelly

* School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, Ireland. E-mail: m.jansen@ucc.ie

Large scale cultivation of Lemnaceae is of growing interest. Objectives differ, and include waste water remediation, starch production (for bioethanol), feed and protein production or combinations of these. Further exciting perspectives are the generation of human food (see Shoham, 2016; Appenroth et al., 2017), including fortified foods, and biopharmaceutics. For most applications it is important to control the composition of the duckweed population. Different species and/or clones of Lemnaceae are differently suited for particular applications. In a previous version of this Newsletter, the group from Qingdao reported on a *Landoltia punctata* clone with particularly strong starch production, and another clone with relatively high cadmium tolerance (Quingdao Duckweed Research Team, 2015). Work here at University College Cork (UCC, Ireland) has shown marked differences between different clones of *Lemna minor* and of *Lemna minuta*. The relative growth rates of *L. minor* clones varied by as much as a factor 2.5, while those of *L. minuta* varied by roughly a factor 2. PhD student Simona Paolacci together with project student Ronan Bonfield could link high relative growth rates in some clones to high net assimilation rates (weight gain per leaf area). However, leaf area ratios (frond area as a function of weight) also varied dramatically between clones. Such variations within natural populations appear very common. Indeed, variations in relative growth rate, doubling time and relative yield have been reported across most taxa of Lemnaceae (Ziegler et al., 2015), indicating the substantial potential for "crop improvement" through selection and/or breeding. Other interesting traits include salt tolerance (Sree et al., 2015) and growth under low (winter) temperatures. At UCC (mild Irish climate!) *Lemna minor* displays growth throughout much of the winter, while *Lemna minuta* does not. We are only starting to explore this phenomenon of low temperature growth. However, it will be of practical relevance for large scale, outdoor cultivation of Lemnaceae in the temperate climate zone. Clearly, selecting the right species and/or clone(s) can potentially give a commercial Lemnaceae enterprise an advantage. Therefore, a future can be envisaged whereby growers will only grow selected clones, which optimally perform under the local growth conditions.

This perspective triggers an important question, how to prevent the mixing of "undesirable" species or clones with selected Lemnaceae when these are grown under outdoor conditions. Perhaps more fundamentally, this triggers the question how do duckweeds disperse?

A male mallard duck (Anas platyrhynchos), which has just left a Lemna-covered pond, takes some "hitch-hiking" duckweed across land on a foraging trip (image © Roy Battell).

Elias Landolt dedicated an entire section in his monographs (Landolt 1986) to dispersal. Landolt (1986) listed dispersal by wind, waves and currents, and mentions the example of dispersal of Wolffia fronds by a tornado. However, in many cases it is animals that transport fronds between waterbodies. Landolt (1986) lists beavers, rats, muskrats, racoons and water birds.

Evidence is often anecdotal, and refers to epizoochorous transport, i.e. transport of plants attached to the outside of the animal, as opposed to endozoochorous transport through the gut of the transporting animal[1]. In the case of Lemnaceae, it has been argued that rapid drying out of fronds will limit the distance of dispersal, and that the frequency of transport will be low. However, the reality appears different.

We noted that the rain water butts near the UCC glasshouses were frequently colonised by *Lemna minor*, which at that stage was being cultured in a number of mini-ponds some 50 meters away. Neil Coughlan was at this stage an MSc student, and the observation of *L. minor* dispersal made him doubt the assumption that transfer of Lemnaceae is a "rare" process, while simultaneously questioning the mechanism of the actual transfer of fronds. To study Lemnaceae dispersal, Neil developed a simple system whereby a bowl with floating *L. minor* was perched on top of a wooden fencing stake. Further bowls containing just water were perched on other stakes short distances away. Quite surprisingly, Neil observed a total of 67 separate dispersal events (transfer of at least one frond) over a period of 20 weeks, and across 6 replicate stake and bowl structures. In total 156 colonies comprising 317 fronds were found to be transferred to receiving bowls in a relatively short period (full details see Coughlan et al., 2017). Exclusion of rodents using "funnel-shaped" structures across stakes did not affect dispersal, nor did dispersal correlate with wind speed or direction. Rather, film recorded using digital camera traps indicated an association between garden-bird visits and *L. minor* dispersal. The role of birds in Lemnaceae dispersal was subsequently demonstrated in experiments with domesticated mallard ducks (*Anas platythynchos*), kept in an enclosure containing water baths with and without Lemna-cover. The Lemnaceae were found to be effectively dispersed

1 Endozoochorous transport of seeds has been well documented, however in the case of Lemnaceae it is not clear whether seeds survive exposure to gut-conditions (Landolt 1986).

between baths by the mallards. Clearly, *L. minor* is a highly mobile species. The question remains, however, over what distances Lemnaceae can be dispersed, a question that focusses heavily on desiccation tolerance of the plants.

There is a considerable amount of data on desiccation of various Lemnaceae, for example Landolt (1986) lists Lemnaceae survival times outside the water of between 20 minutes and 2.5 hours. However, these data tell only half the story as the microclimate between the feathers of a bird also needs to be considered. *Lemna minuta* taken out of the aquatic medium was found to have lost viability after just 90 minutes at a Relative Humidity (RH) of 44% and a temperature of 21˚C (Coughlan et al., 2015). At a slightly higher RH of 58% (T = 23˚C) *Lemna minuta* still displayed some viability after 4 hours out of the aquatic medium (Coughlan et al., 2015). So, how do these experimental conditions compare to the microclimate between feathers of a duck? Neil Coughlan's research showed that the RH is around 65% and the temperature 23˚C between the feathers near the posterior neck of a mallard duck. Near the inner crural[2], the RH is even higher at around 77% with a temperature of 24˚C. Interestingly, the downy feathers of the inner crural were also found to retain entangled Lemnaceae fronds more effectively than areas of less downy plumage, such as the back of the neck. All in all, we reckon that Lemna minuta can be entangled between feathers, and survive flights of up to four hour's duration. Given an average speed for mallards of 65 km/h-1, we argue that duckweed dispersal over distances of up to 250km is realistic, although much shorter distances (< 50km) are likely more common.

Lemna minor (the bigger fronds) in competition with large numbers of *Lemna minuta* colonies in a mesocosm at University College Cork, Ireland.

Lemna minuta is an alien invasive species in Europe, co-occurring with native *Lemna minor* . American *L. minuta* was first reported in France in the 1960s, and has since spread throughout much of Europe including Ireland and Britain in the west, the Netherlands and Germany in the north, Poland and Hungary in the east, and Italy and Malta in the south. Thus, substantial distances have been covered by the species, including crossing of the Mediterranean, North and Celtic seas, and mountain ridges such as the Pyrenees and the Alps. This once more underlines the mobility of Lemnaceae. Although it hasn't been proven that birds played a role in dispersal of *Lemna minuta* throughout Europe, it seems likely that birds were at least a contributory factor in this process.

So where does that leave the duckweed industry? There are two practical considerations for Lemnaceae cultivation systems:

2 Inner crural refers to the upper part of the leg.

(1) preventive steps need to be taken if one wants to avoid bird-mediated[3] contamination of an outdoor Lemnaceae culture (e.g. dilution of a selected clone by non-selected, native clones)

(2) preventive steps need to be taken to avoid introduction of selected alien species or clones into the local environment. At present, substantial efforts are involved in control of *Landoltia punctata* in Florida USA, where this is an alien, invasive species. Similarly, *Lemna minuta* is the focus of management efforts in various European countries. There is absolutely no evidence that the introduction of *L. punctata* in Florida, or *L. minuta* in Europe is associated with cultivation of these species by the Lemnaceae industry. Nevertheless, the industry needs to adopt a responsible approach when cultivating alien species of Lemnaceae, and prevent their spread in to the surrounding environment in order to maintain the positive public perception of duckweed applications as being eco-friendly and sustainable.

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3 Rodents, as well as implements such as boots, tools and boats also need to be considered.

Student Spotlight: Ömer Faruk Coşkun

Email: ofcoskun1@hotmail.com

I always admired aquatic habitats and plants and I am all the time interested in researches and articles on these natural topics. Being a biologist allowed me to do independent studies. I graduated from Biology Department of Erciyes University (Turkey). My graduation thesis at the university was water quality parameters of wetlands. I started to a graduate study at Hydrobiology Department in 2013 and then interested in stress physiology subjects. I initially performed studies on toxic substance-induced behavioral changes in fish, changes in blood parameters and histopathology of fish. I also investigated the changes in blood parameters of fish living in still and running water. Then, I worked on heavy metal ratios in water, fish and plants of aquatic systems, and tried to elucidate the relationships between them. I tended then to aquatic plant ecophysiology and phytoremediation studies. Throughout these studies, I worked on heavy metal toxicity in *Lemna minor*, *Ceratophillium demercum* and *Nasturtium officinale* plants. I performed researches about potential use of duckweeds as a bio-indicator and about their phytoremediation characteristics.

Then I started doing my Ph.D. studies under the supervision of Assoc.Prof.Dr. Didem Aydin, who was my advisor also in my undergraduate and graduate thesis. I already had several studies on stress-induced changes in morphologic and physiological characteristics of duckweeds. Now, I want to identify genotoxic impacts. While I was searching for stress-induced genotoxicity, I found myself now in molecular biology. I tended to molecular works throughout my Ph.D. study and took classes on marker analyses, sequence analyses and new-generation technologies. Then, I met Prof.Dr. Osman Gülşen for advanced studies on molecular analyses and started doing a second Ph.D. at Horticulture Department of Agricultural Faculty. With his aid, I quite enriched my theoretical and practical knowledge and experiences in molecular biology. Now, I shaped in mind about what I can do on duckweeds in molecular sense and what I can do on duckweed cultivation in agricultural sense. As long as I searched through literature on duckweeds, I noticed that there weren't any studies carried out about distribution and genetic diversity of duckweeds in Turkey. Then, I decided to start from that point. I also noticed that some molecular markers haven't been used or tested in duckweed samples. Therefore, I started conducting a Ph.D. thesis on molecular characterization and phylogenetic analyses of duckweed species of Turkey. I identified aquatic sites with duckweed populations in large portion of the country and I identified the existing species. Then with different molecular markers, I identified inter and intra-species molecular differences. Within the scope of the same study, I determined the efficiency of several DNA markers which haven't been tested in duckweeds before. I also tested different DNA isolation methods in different duckweed species and identified which isolation method is advantageous with regard to quality and quantity in which species. I also performed some other studies on duckweeds like use of molecular marker methods to identify heavy metal genotoxicity in duckweeds.

Turkey is quite rich in plant diversity and endemic species. Of about ten thousand plant species, three thousands are endemic. Wetlands with great biological diversity also quite widespread in Turkey. Duckweed is called in Turkish as "sumercimeği" (water lentil). Duckweeds are also rich in protein and thus several researchers indicated potential use of them as fish feed, some other researchers pointed out potential use of duckweeds as biofuel. Duckweeds are the fastest growing the smallest flowering plants, therefore, they are used as a model plant in various studies. I comprehended the significance of duckweeds after I met with them and had comprehensive literature review about them. I have completed quite small portion of the works I wished to carry out on duckweeds. I believe there are a lot of works to be done on duckweeds. My ultimate target is to provide outcomes for the benefit of both the society and the nature. I always follow duckweeds-related articles, papers, symposium and conferences curiously. I love my profession and I wish to do post-doctoral studies abroad to improve my knowledge and experiences on duckweeds.

Useful Methods 7: Cultivation, sterilization and stock cultivation of duckweeds; an update

K. Sowjanya Sree[1], Klaus-J. Appenroth[2]

[1]Central University of Kerala, Department of Environmental Science, Padanakkad 671314, Kerala, India; Email: ksowsree@gmail.com

[2]Friedrich Schiller University Jena, Institute of General Botany and Plant Physiology, 07743 Jena, Germany; Email: Klaus.Appenroth@uni-jena.de

Over the time methods develop. And, here are a few updates to standard methods in handling duckweed plants that were published in the previous issues of Duckweed Forum.

1. Cultivation of duckweed

See "Useful Methods 3: Media for in vitro cultivation of duckweed" In: Duckweed Forum 3 (4), 180-186 (2015) in http://lemnapedia.org/wiki/Duckweed_Forum#2015-10

Recently, we observed that there is hardly any difference in the growth rate of duckweed grown in N-medium and modified Steinberg medium (i.e., enhanced concentration of FeNaEDTA in comparison to ISO 20070 protocol). Hence, we recommend the use of any of these two media for routine cultivation of duckweeds.

2. Sterilization of Duckweeds

See "Useful Methods 2: Sterilization of duckweed" In: Duckweed Forum 3(3), 90-91 (2015) in http://lemnapedia.org/wiki/Duckweed_Forum#2015-07

Sterilizing some of the recently collected samples from India,we had learnt that it is useful to apply a rather medium concentration of bleach, i.e. between 4 and 6 % "Eau de Javel" or "DanKlorix" (2.4 or 2.8 % NaOCl, respectively) and to extend the time of treatment until signs of damage can be seen visually in the fronds. Usually, three different time points of treatment with bleach were used to sterilize the fronds. This makes it possible to adapt to the morphology of the clone under treatment. Nevertheless, the treatment time points as suggested in the previous report might be helpful for orientation.

When plants are freshly collected from nature and grown under in vitro condition, contamination by algae is often the most urgent problem. When it is not controlled, algae may overgrow and finally out-compete duckweeds. In such cases, we sterilized the plants for only 2 min and then transferred them to nutrient media (N-medium or Steinberg medium) without sugar. However, it is not certain that the plant samples are free of any contamination by bacteria or fungi. The plants that recover from sterilization with bleach solution and, free of algae-contamination, can then be used for further more stringent sterilization.

After stringent sterilization, plants have to be inoculated directly into nutrient medium containing sugar. In the presence of bacteria or fungi these microorganism will grow very fast and produce visible signs like turbidity or coloration of the medium. In our previous report we mentioned that the original concentration of glucose (50 mM, 9.91 g glucose-hydrate per litre) as a source of sugar in the medium was too high for several duckweed species. We compared 25 mM fructose, 25 mM glucose and 25 mM sucrose and we suggest using 25 mM glucose.

3. Stock cultivation of duckweed

See "Useful Methods 4: Stock cultivation of Duckweed" In: Duckweed Forum 4 (1), 216-217 (2016) in http://lemnapedia.org/wiki/Duckweed_Forum#2016-01

We adjusted the GELRITE (e.g. Carl Roth GmbH, Karlsruhe, Germany) concentration for solidification of media now to 0.4 % instead of 0.45 %. This agent has the advantage over Agar-Agar that it does not dry out easily over the time.

Stock cultivation of *Lemna minor* 5500 on gelrite and *Spirodela polyrhiza* 9509 in liquid N medium. Total genome sequences were reported for 5500 by A. van Hoeck et al. (2015) and 9509 by T. Michael et al (ePub 2016).

In the last few months we compared 25 mM glucose and 25 mM sucrose in gelrite and Agar media. It turned out that 25 mM glucose is superior to 25 mM sucrose for long-term cultivation on gelrite. Fronds on sucrose-containing gelrite died much earlier than on glucose–containing gelrite.

Sugars are used in Agar or gelrite during stock cultivation to recognize possible infections that may not be uncovered without sugar. However, sugar accelerates clearly the senescence of fronds. This is against the interest of the stock cultivation of plants that is to keep the plants alive as long as possible before the next round of inoculation is required. Instead of keeping the plants on two or three different gelrite flasks with sugar, we decided now to have for each clone one flask with 25 mM glucose in order to recognize probable infections, and one flask without sugar, in which the plants will be vital for a much longer time. This is also helpful to save the clones when an infection would contaminate the plants.

From the data base

Biotechnology

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

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

FUEL 185: 229-235 (2016)

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

Activated carbons for the hydrothermal upgrading of crude duckweed bio-oil

Duan, PG; Zhang, CC; Wang, F; Fu, J; Lu, XY; Xu, YP; Shi, XL

CATALYSIS TODAY 274: 73-81 (2016)

This study examined a two-stage (noncatalytic pretreatment followed by catalytic upgrading) hydrothermal processing of crude bio-oil produced from the hydrothermal liquefaction of duckweed. The activities of six activated carbons (ACs)-pine wood AC, coconut shell AC, bamboo stem AC, apricot pit AC, peach pit AC, and coal AC-toward the deoxygenation and denitrogenation of the pretreated duckweed bio-oil were determined in supercritical water at 400 degrees C for 1 h with the addition of 6 MPa of H-2 and 10 wt% AC. All of the ACs exhibited activity similar to Ru/C toward the denitrogenation and deoxygenation of the pretreated duckweed bio-oil. Of the ACs tested, bamboo stem AC produced an upgraded bio-oil with the highest yield (76.3 wt%), the highest fraction (90.13%) of material boiling below 350 degrees C, and the highest energy density (44.1 MJ/kg). Decreased ash and acidic groups in the pre-treated AC disfavored the production of upgraded bio-oil but aided denitrogenation and desulfurization. The ACs are suspected to leach ions and weak acids into the reaction solution, which would catalyze denitrogenation and desulfurization. The gases mainly consisted of unreacted H-2, CO2 and CH4 together with small amounts of CxHy (x <= 5, y <= 12) hydrocarbon gases produced from the cracking of the upgraded bio-oil.

Positive effects of duckweed polycultures on starch and protein accumulation

Li, Y; Zhang, FT; Daroch, M; Tang, J

BIOSCIENCE REPORTS 36, Part: 5, Article Number: e00380 (2016)

The effect of duckweed species composition (Lemna aequinoctialis 5505, Landoltia punctata 5506 and Spirodela polyrhiza 5507) in polyculture and monoculture on biomass and starch/protein content were investigated at different levels of temperature, light intensity, nitrogen and phosphorus concentrations. The three growth parameters significantly affect duckweed biomass accumulation. Different combinations of duckweed species greatly varied in starch/protein content. Although all the polycultures showed a median relative growth rate and the majority of the polycultures showed a median and starch/protein content as compared with their respective monocultures, some of the polycultures were found to promote the accumulation of starch/protein at different growth conditions. These findings indicated that proper combination of duckweed species could facilitate desirable biomass accumulation and improve biomass quality. The present study provides useful references for future large-scale duckweed cultivation.

Ecology

Does the alien *Lemna minuta* show an invasive behavior outside its original range? Evidence of antagonism with the native *L. minor* in central Italy

Ceschin, S; Abati, S; Leacche, I; Iamonico, D; Iberite, M; Zuccarello, V

INTERNATIONAL REVIEW OF HYDROBIOLOGY 101: 173-181 (2016)

The American duckweed Lemna minuta Kunth is considered an invasive species in several European countries (e.g., Belgium, Germany, Hungary, Ireland, Italy), but its invasiveness outside its original range remains poorly studied. We therefore analyse: (i) the speed with which L. minuta has spread throughout Europe; and (ii) its capability to compete with L. minor L., the most common European native duckweed. Based on literature data, the increasing number of observations of L. minuta since it was reported for the first time in France (1966) indicated the wide extent reached by this species in most of Europe, highlighting its high capacity to colonize new areas. By sampling Lemna populations from 41 stations in standing waters in Central Italy, it emerged that the frequency of the two species was similar, but the alien species was more abundant than the native one, showing generally higher coverages, and predominating in mixed Lemna populations. A negative correlation between L. minuta and L. minor was observed in our study. This relationship was not explained by different ecological requirements, since the two species responded similarly to the main environmental gradient, but was linked to an antagonistic dynamism between both species. A multitemporal analysis, based on the comparison of Lemna coverage data recorded at the same stations at two different times (last vs. first record), revealed that L. minuta had appeared where it had been absent previously and that its abundance had increased to the detriment of L. minor, which in some cases disappeared within a few years. The arrival of L. minuta in a site can be considered a real threat to conservation of the native plant diversity due to its competition with the congeneric L. minor.

Functional response (FR) and relative growth rate (RGR) do not show the known invasiveness of *Lemna minuta* (Kunth)

Van Echelpoel, W; Boets, P; Goethals, PLM

PLOS ONE 11,Issue: 11, Article Number: e0166132

Growing travel and trade threatens biodiversity as it increases the rate of biological invasions globally, either by accidental or intentional introduction. Therefore, avoiding these impacts by forecasting invasions and impeding further spread is of utmost importance. In this study, three forecasting approaches were tested and combined to predict the invasive behaviour of the alien macrophyte Lemna minuta in comparison with the native Lemna minor: the functional response (FR) and relative growth rate (RGR), supplemented with a combined biomass-based nutrient removal (BBNR). Based on the idea that widespread invasive species are more successful competitors than local, native species, a higher FR and RGR were expected for the invasive compared to the native

species. Five different nutrient concentrations were tested, ranging from low (4 mgN. L-1 and 1 mgP. L-1) to high (70 mgN. L-1 and 21 mgP. L-1). After four days, a significant amount of nutrients was removed by both Lemna spp., though significant differences among L. minor and L. minuta were only observed at lower nutrient concentrations (lower than 17 mgN. L-1 and 6 mgP. L-1) with higher nutrient removal exerted by L. minor. The derived FR did not show a clear dominance of the invasive L. minuta, contradicting field observations. Similarly, the RGR ranged from 0.4 to 0.6 d(-1), but did not show a biomass-based dominance of L. minuta (0.5 +/- 0.1 d(-1) versus 0.63 +/- 0.09 d(-1) for L. minor). BBNR showed similar results as the FR. Contrary to our expectations, all three approaches resulted in higher values for L. minor. Consequently, based on our results FR is sensitive to differences, though contradicted the expectations, while RGR and BBNR do not provide sufficient power to differentiate between a native and an invasive alien macrophyte and should be supplemented with additional ecosystem-based experiments to determine the invasion impact.

Interaction with other organisms

Two-stage phyto-microremediation of tannery effluent by *Spirodela polyrrhiza* (L.) Schleid. and chromium resistant bacteria

Singh, A; Vyas, D; Malaviya, P

BIORESOURCE TECHNOLOGY 216: 883-893 (2016)

Two-stage sequential treatment of tannery effluent was conducted employing a wetland plant, Spirodela polyrrhiza (L.) Schleid., and chromium (Cr) resistant bacterial strains. The bacterial strains were isolated from Cr-enriched environmental matrices and rhizosphere of Spirodela polyrrhiza. The phytorhizoremediation of tannery effluent by Spirodela and its rhizospheric bacteria (Cellulomonas biazotea APBR1-6, Bacillus safensis APBR2-12, Staphylococcus warneri APBR3-5, Microbacterium oleivorans APBR2-6), followed by microremediation by Cr resistant bacteria (Micrococcus luteus APBS5-1, Bacillus pumilus APBS5-2, Bacillus flexus APBE3-1, Virgibacillus sediminis APBS6-1) resulted in reduction of pollution parameters [COD (81.2%), total Cr (97.3%), Cr(VI) (99.3%), Pb(II) (97.0%), Ni (95.7%)]. The LC-MS analysis showed that many pollutants detected in untreated tannery effluent were diminished after bioremediation or long chains of alcohol polyethoxylates viz. C18EO6 in untreated effluent were broken down into smaller unit of alcohol polyethoxylate (+HHO-[-CH2-CH2-O-]-H), indicating that bacteria and Spirodela polyrrhiza, along with its rhizospheric associates utilized them as carbon and energy source.

Molecular Biology

Synthesis of the (9R,13R)-isomer of LDS1, a flower-inducing oxylipin isolated from *Lemna paucicostata*

Takayasu, Y; Ogura, Y; Towada, R; Kuwahara, S

BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY 80: 1459-1463 (2016)

The first synthesis of the (9R,13R)-stereoisomer of LDS1, a flower-inducing oxylipin isolated from Lemna paucicostata, has been achieved from a known allylic alcohol by a seven-step sequence that involves the Horner-Wadsworth-Emmons olefination to construct its full carbon framework and an enzymatic hydrolysis of a penultimate methyl ester intermediate to provide the target molecule.

Physiology

Effects of simulated microgravity on the performance of the duckweeds *Lemna aequinoctialis* and *Wolffia globosa*

Yuan, JX; Xu, KY

AQUATIC BOTANY 137: 65-71 (2017)

Duckweed is considered to be one of the most attractive higher plants to provide the life support requirements of the crew in long-duration space missions. Two species of duckweed, Lemna aequinoctialis and Wolffia globosa, were comparatively studied with focus on the effects of simulated microgravity conditions. A significant enhancement of plant relative growth rate (RGR) was observed under simulated microgravity conditions. The mean RGR of L. aequinoctialis was 32% increased, and W. globosa was 12% increased compared to control. Light and electron microscope analyses showed that the mesophyll cells of duckweed fronds were loosely arranged and many intercellular air spaces enclosed by mesophyll cells were observed in reaction to simulated microgravity, and few starch grains were detected in root tip cells in L. aequinoctialis under simulated microgravity conditions. For L aequinoctialis, the starch content was significantly increased under simulated microgravity conditions. Thus, the starch content of W. globosa under simulated microgravity conditions was kept stable during all growth periods. This pattern might be associated with the different expression change of the rate-limiting gene APL in regulating starch levels. Taking into consideration of the duckweed growth, ultra-structure and starch content changes, we considered that simulated microgravity conditions do not have a significant adversely affect on the duckweed growth.

Insights into the structural and physicochemical properties of small granular starches from two hydrophyte duckweeds, *Spirodela oligorrhiza* and *Lemna minor*

Chen, L; Yu, CJ; Ma, YB; Xu, H; Wang, SM; Wang, Y; Liu, XX; Zhou, GK

CARBOHYDRATE RESEARCH 435: 208-214 (2016)

The structure and physicochemical properties of starches from two hydrophyte duckweeds, Spirodela oligorrhiza and Lemna minor, were investigated and compared in this study. The amylose content and average size of starches were determined to be 20.85%, 4.70 mu m and 27.77%, 6.17 mu m for Spirodela oligorrhiza and Lemna minor, respectively. The average chain length of two duckweed starches was measured to be around DP 28. The chain length distribution was observed to be greatly different from other reported starches for the high proportion of long chains (DP >= 37) over 50%. Wide-angle X-ray diffraction profiles of the two starch samples displayed typical B-type diffraction pattern. The gelatinization enthalpy-changes (Delta H-gel) of two starch samples was about 10.40 J/g for two duckweed starches. The present results suggested the potential utilization of small granular starches from duckweed in functional foods and dietary supplement products.

Content of biogenic amines in *Lemna minor* (common duckweed) growing in medium contaminated with tetracycline

Baciak, M; Sikorski, L; Piotrowicz-Cieslak, AI; Adomas, B

AQUATIC TOXICOLOGY 180: 95-102 (2016)

Aquatic plants are continuously exposed to a variety of stress factors. No data on the impact of antibiotics on the biogenic amines in duckweed (Lemna minor) have been available so far, and such data could be significant, considering the ecological role of this plant in animal food chains. In the tissues of control (non stressed) nine-day-old duckweed, the following biogenic amines were identified: tyramine, putrescine, cadaverine, spermidine and spermine. Based on the tetracycline contents and the computed EC values, the predicted toxicity units have been calculated. The obtained results demonstrated phytoxicity caused by tetracycline in relation to duckweed growth rate, yield and the contents of chlorophylls a and b. The carotenoid content was not modified by tetracycline. It was found that tetracycline as a water pollutant was a stress factor triggering an increase in the synthesis of amines. Tetracycline at 19, 39 and 78 p.M concentrations increased biogenic amine synthesis by 3.5 times. Although the content of tyramine increased fourteen times with the highest concentration of the drug (and of spermidine only three-fold) the increase of spermidine was numerically the highest. Among the biogenic amines the most responsive to tetracycline were spermine and tyramine, while the least affected were putrescine and spermidine.

Despite putrescine and spermidine being the least sensitive, their sum of contents increased five-fold compared to the control. These studies suggest that tetracycline in water reservoirs is taken up by L. minor as the antibiotic clearly modifies the metabolism of this plant and it may likely pose a risk.

Phytoremediation

Potential of duckweed (*Lemna minor*) for removal of nitrogen and phosphorus from water under salt stress

Liu, CG; Dai, Z; Sun, HW

JOURNAL OF ENVIRONMENTAL MANAGEMENT 187: 497-503 (2017)

Duckweed plays a major role in the removal of nitrogen (N) and phosphorus (P) from water. To determine the effect of salt stress on the removal of N and P by duckweed, we cultured Lemna minor, a common species of duckweed, in N and P-rich water with NaCl concentrations ranging from 0 to 100 mM for 24 h and 72 h, respectively. The results show that the removal capacity of duckweed for N and P was reduced by salt stress. Higher salt stress with longer cultivation period exerts more injury to duckweed and greater inhibition of N and P removal. Severe salt stress (100 mM NaCl) induced duckweed to release N and P and even resulted in negative removal efficiencies. The results indicate that L. minor should be used to remove N and P from water with salinities below 75 mM NaCl, or equivalent salt stress.

Bioaccumulation of Uranium and Thorium by *Lemna minor* and *Lemna gibba* in Pb-Zn-Ag tailing water

Sasmaz, M; Obek, E; Sasmaz, A

BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 97: 832-837 (2016)

This study focused on the ability of Lemna minor and Lemna gibba to remove U and Th in the tailing water of Keban, Turkey. These plants were placed in tailing water and individually fed to the reactors designed for these plants. Water and plant samples were collected daily from the mining area. The plants were ashed at 300A degrees C for 1 day and analyzed by ICP-MS for U and Th. U was accumulated as a function of time by these plants, and performances between 110 % and 483 % for L. gibba, and between 218 % and 1194 % for L. minor, were shown. The highest Th accumulations in L. minor and L. gibba were observed at 300 % and 600 % performances, respectively, on the second day of the experiment. This study indicated that both L. gibba and L. minor demonstrated a high ability to remove U and Th from tailing water polluted by trace elements.

Removal of Cr, Ni and Co in the water of chromium mining areas by using *Lemna gibba* L. and *Lemna minor L*

Sasmaz, A; Dogan, IM; Sasmaz, M

WATER AND ENVIRONMENT JOURNAL 30: 235-242 (2016)

This study investigated the use of Lemna gibba and Lemna minor plant species to absorb Cr, Ni and Co from Alacakaya mining area water. Lemna gibba and L. minor were separately placed to feed into two reactors. Water and plant samples were collected for eight consecutive days, and the pH, electric conductivity and temperature of the water were measured. The plants were washed, dried and burned at 300 degrees C for 24 h in a drying oven. The samples were then analysed by ICP-MS (inductively coupled plasma mass spectroscopy) for concentrations of Cr, Ni and Co, which were 1.2, 0.9 and 0.5 gL(-1) respectively. On Day 8, the determined uptake of L. gibba and L. minor were: 196 and 398% for Cr; 307 and 1473% for Ni; and 166 and 223% for Co respectively. Lemna gibba and L. minor were thus effective in absorbing Cr, Ni and Co from mining water.

Biosorption of reactive blue 19 dye using *Lemna minor*: Equilibrium, kinetic and thermodynamic studies

Balarak, D; Mostafapour, FK; Azarpira, H

BIOSCIENCE BIOTECHNOLOGY RESEARCH COMMUNICATIONS 9: 558-566 (2016)

Dyes are the main pollutants existing in wastewater of textile industries. This paper presents the sorption studies of Reactive Blue 19 (RB19) dye by the Lemna minor. The effect of different parameters like pH, adsorbent dose, contact time, temperature and initial dye concentrations were investigated. The biosorption data have been analysed using Langmuir, Freundlich and Temkin isotherms. The equilibrium uptake capacity was increase from 7.12 mg/g to 46.51 mg/g, when increasing the dye concentration from 25 mg/L to 200 mg/L. The equilibrium data were best represented by the Langmuir isotherm. The adsorption kinetics were found to follow the pseudo-second-order kinetic model. Thermodynamic parameters such as Delta Go, Delta Ho and Delta So have also been evaluated and sorption process was feasible, spontaneous and exothermic in nature. The results indicate that L. minor is a suitable adsorbent for the adsorption of textile dyes.

Phytotoxicity

Comparing the acute sensitivity of growth and photosynthetic endpoints in three *Lemna species* exposed to four herbicides

Park, J; Brown, MT; Depuydt, S; Kim, JK; Won, DS; Han, T

ENVIRONMENTAL POLLUTION 220: 818-827 (2017)

An ecological impact assessment of four herbicides (atrazine, diuron, paraquat and simazine) was assessed using the aquatic floating vascular plants, Lemna gibba, Lemna minor and Lemna paucicostata as test organisms. The sensitivity of several ecologically relevant parameters (increase in frond area, root length after regrowth, maximum and effective quantum yield of 1,511 and maximum electron transport rate (ETRmax), were compared after a 72 h exposure to herbicides. The present test methods require relatively small sample volume (3 mL), shorter exposure times (72 h), simple and quick analytical procedures as compared with standard Lemna assays. Sensitivity ranking of endpoints, based on EC50 values, differed depending on the herbicide. The most toxic herbicides were diuron and paraquat and the most sensitive endpoints were root length (6.0-123 mu g L-1) and ETRmax (4.7-10.3 mu g L-1) for paraquat and effective quantum yield (6.8-10.4 mu g L-1) for diuron. Growth and chlorophyll a fluorescence parameters in all three Lemna species were sensitive enough to detect toxic levels of diuron and paraquat in water samples in excess of allowable concentrations set by international standards. CV values of all EC(50)s obtained from the Lemna tests were in the range of 2.8-24.33%, indicating a high level of repeatability comparable to the desirable level of <30% for adoption of toxicity test methods as international standards. Our new Lemna methods may provide useful information for the assessment of toxicity risk of residual herbicides in aquatic ecosystems.

Bioaccumulation and toxicity assessment of irrigation water contaminated with boron (B) using duckweed (*Lemna gibba* L.) in a batch reactor system

Turker, OC; Yakar, A; Gur, N

JOURNAL OF HAZARDOUS MATERIALS 324: 151-159, Part: B (2917)

The present study assesses ability of Lemna gibba L. using a batch reactor approach to bioaccumulation boron (B) from irrigation waters which were collected from a stream in largest borax reserve all over the world. The important note that bioaccumulation of B from irrigation water was first analyzed for first time in a risk assessment study using a Lemna species exposed to various B concentrations. Boron toxicity was evaluated through plant growth and biomass production during phytoremediation process. The result from the present experiment indicated that L. gibba was capable of removing 19-63% B from irrigation water depending upon contaminated level or initial concentration. We also found that B was removed from aqueous solution following pseudo second order kinetic model and Langmuir isotherm model better fitted equilibrium obtained for B phytoremediation. Maximum B accumulation in L. gibba was determined as 2088 mg kg(-1) at average inflow B concentration 17.39 mg L-1 at the end of the experiment. Conversely, maximum bioconcentration factor obtained at lowest inflow B concentrations were 232 for L. gibba. The present study suggested that L. gibba was very useful B accumulator, and thus L. gibba-based techniques could be a reasonable phytoremediation option to remove B directly from water sources contaminated with B.

Toxicity of copper oxide nanoparticles on *Spirodela polyrrhiza*: assessing physiological parameters

Khataee, A; Movafeghi, A; Mojaver, N; Vafaei, F; Tarrahi, R; Dadpour, MR

RESEARCH ON CHEMICAL INTERMEDIATES 43: 927-941 (2017)

The surface characteristics of nanoparticles cause their influx into the environment and lead to their interaction with fungi, algae, and plants. In the present study, the toxic effects of copper oxide nanoparticles were studied on the higher aquatic plant Spirodela polyrrhiza. Copper oxide nanoparticles were synthesized using green sono-chemistry and their surface specifications were determined using XRD and SEM. The entrance and uptake of CuO nanoparticles in the roots of S. polyrrhiza was confirmed using fluorescence microscopy. The toxicity of CuO nanoparticles on S. polyrrhiza was investigated by measuring the growth rate (relative frond number), enzymatic activities (peroxidase, superoxide dismutase, and catalase) and content of photosynthetic pigments. In all experiments, the negative effects of CuO nanoparticles on the growth of S. polyrrhiza were confirmed by means of growth and enzymatic and pigment assays. Accordingly, significant changes in antioxidant enzyme activity were achieved. Catalase, peroxidase, and superoxide dismutase activity were increased due to the plant's defence system for scavenging the reactive oxygen species. In addition, relative frond number and chlorophyll content were reduced owing to possible phytotoxicity generated by CuO nanoparticles.

Impact of ionic and nanoparticle speciation states of silver on light harnessing photosynthetic events in *Spirodela polyrhiza*

Shabnam, N; Sharmila, P; Pardha-Saradhi, P

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 19: 80-86 (2017)

Owing to wide range of applications, nanotechnology is growing expeditiously. Likely negative impact of nanoparticles (NPs), which are inevitably released into our surroundings, on living organisms is of growing concern. Findings presented here are outcome of investigations carried out to evaluate the impact of ionic and NP speciation states of silver on light harnessing photosynthetic events in Spirodela polyrhiza fronds. Fronds exposed to ionic speciation state showed significant decline in PS (photosystem) II efficiency (Fv/ Fm; variable fluorescence/maximal fluorescence), while those exposed to silver nanoparticles (Ag-NPs) showed marginal decline. Accordingly, decline in amplitude of Chl a fluorescence transients was sharper in fronds treated with Ag+ than those treated with Ag-NPs. Of the various phases Chl a fluorescence transient, J-I phase [which reflects reduction of plastoquinone (PQ) pool] was most sensitive to both Ag+ and Ag-NPs. Phenomenological yield models, built using Biolyzer software, revealed that fronds exposed to AgC possessed significantly lower potential to trap and harness absorbed light energy for photochemical reactions than those exposed to Ag-NPs. Accordingly, dissipation of absorbed light energy as heat was significantly higher in fronds exposed to AgC than those exposed to Ag-NPs. These findings revealed that NP speciation state of silver is significantly less toxic to light harnessing photosynthetic machinery of S. polyrhiza, compared to ionic speciation state.

Getting more ecologically relevant information from laboratory tests: Recovery of *Lemna minor* after exposure to herbicides and their mixtures

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

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

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

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

Gomes, MP; Juneau, P

ENVIRONMENTAL POLLUTION 218: 402-409 (2016)

We investigated the physiological responses of Lemna minor plants exposed to glyphosate. The deleterious effects of this herbicide on photosynthesis, respiration, and pigment concentrations were related to glyphosate-induced oxidative stress through hydrogen peroxide (H2O2) accumulation. By using photosynthetic and respiratory electron transport chain (ETC) inhibitors we located the primary site of reactive oxygen species (ROS) production in plants exposed to 500 mg glyphosate l(-1). Inhibition of mitochondrial ETC Complex I by rotenone reduced H2O2 concentrations in glyphosate-treated plants. Complex HI activity was very sensitive to glyphosate which appears to act much like antimycin A (an inhibitor of mitochondrial ETC Complex III) by shunting electrons from semiquinone to oxygen, with resulting ROS formation. Confocal evaluations for ROS localization showed that ROS are initially produced outside of the chloroplasts upon initial glyphosate exposure. Our results indicate that in addition to interfering with the shikimate pathway, glyphosate can induce oxidative stress in plants through H2O2 formation by targeting the mitochondrial ETC, which would explain its observed effects on non-target organisms.

Arsenic toxicity in the water weed *Wolffia arrhiza* measured using Pulse Amplitude Modulation Fluorometry (PAM) measurements of photosynthesis

Ritchie, RJ; Mekjinda, N

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 132: 178-185 (2016)

Accumulation of arsenic in plants is a serious South-east Asian environmental problem. Photosynthesis in the small aquatic angiosperm Wolffia arrhiza is very sensitive to arsenic toxicity, particularly in water below pH 7 where arsenite (As (OH)(3)) (AsIII) is the dominant form; at pH > 7 AsO42- (As (V) predominates). A blue-diode PAM (Pulse Amplitude Fluorometer) machine was used to monitor photosynthesis in Wolffia. Maximum gross photosynthesis (Pg(max)) and not maximum yield (Y-max) is the most reliable indicator of arsenic toxicity. The toxicity of arsenite As(III) and arsenate (H2AsO42-) As (V) vary with pH. As(V) was less toxic than As(III) at both pH 5 and pH 8 but both forms of arsenic were toxic (> 90% inhibition) at below 0.1 mol m(-3) when incubated in arsenic for 24 h. Arsenite toxicity was apparent after 1 h based on Pg(max), and gradually increased over 7 h but there was no apparent effect on Ymax or photosynthetic efficiency (alpha(0)).

Molecular distribution and toxicity assessment of praseodymium by *Spirodela polyrrhiza*

Xu, T; Su, CL; Hu, D; Li, FF; Lu, QQ; Zhang, TT; Xu, QS

JOURNAL OF HAZARDOUS MATERIALS 312: 132-140 (2016)

Aquatic macrophytes are known to accumulate and bioconcentrate metals. In this study, the physiological, biochemical, and ultrastructural responses of Spirodela polyrrhiza to elevated concentrations of praseodymium (Pr), ranging from 0 to 60 mu M, were investigated over 20 d exposure. The results showed that the accumulation of Pr in S. polyrrhiza occurred in a concentration-dependent manner. The accumulation of Pr in biomacromolecules decreased in the order of cellulose and pectin (65-69%), crude proteins (18-25%), crude polysaccharides (6-10%), crude lipids (3%-4%). Significant increases in malondialdehyde (MDA), and decreases in photosynthetic pigment, soluble protein, and unsaturated fatty acids showed that Pr induced oxidative stress. Inhibitory effects on photosystem 11 and the degradation of the reaction center proteins D1 and D2 were revealed by chlorophyll a fluorescence transients, immunoblotting, and damage to chloroplast ultrastructure. Significant increases in cell death were observed in Pr-treated plants. However, S. polyrrhiza can combat Pr induced oxidative injury by activating various enzymatic and non-enzymatic antioxidants. These results will improve understanding of the biological consequences of rare earth elements (REEs) contamination, particularly in aquatic bodies.

Links for Further Reading

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

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

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

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

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