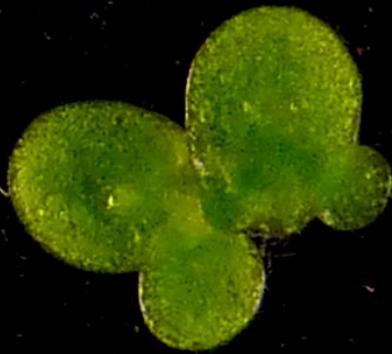


DUCKWEED FORUM



Volume 5 (4), issue 19, pages 85 - 108 (2017)

Lemna minuta 9473



3 mm

Wolffiella oblonga 7343



3 mm

Lemna yungensis 9210



3 mm

Wolffiella repanda 9062



3 mm

Cover page

The cover of this issue of the Duckweed Forum features another four duckweed species from the two genera of *Lemna* and *Wolffiella*. *Lemna minuta* (clone 9473 from the Netherlands), formerly *L. minuscula*, is distributed in Europe and the Americas. It is thought to originate from warmer areas of South America but extended in its area of habitat over more recent years. M. Jansen has reported it as invasive in Ireland and competing there with *L. minor*. During the cold Winter, growth rates of *L. minuta* clones are lower than those of *L. minor* but vice versa during warmer summer times.

Lemna yungensis (clone 9210 from Novia, Bolivia) was discovered by E. Landolt in 1998 in Bolivia and classified mainly by eco-geographic reasons as a new species since it grows on surface of wet rocks. As previously stressed by Landolt, *L. minuta*, *L. yungensis* and *L. valdiviana* belong to the section Uninerves and are very similar to each other morphologically. In a comprehensive plastid barcoding studies using two "universal barcodes" with all 37 species of duckweed, *L. minuta* and *L. valdiviana* are unable to be unambiguously resolved while *L. yungensis* can possibly be distinguished from the other two species by using the psbK-psbI primer set. During the 4th ICDRA, M. Bog et al. will present more recent results from molecular taxonomy that may indicate *L. yungensis* to be *L. valdiviana*. *Wolffiella oblonga* (clone 7343 from Tucuman, Argentina) and *Wolffiella repanda* (clone 9062 from Zimbabwe, Africa) represent species that are also difficult to distinguish from each other by molecular barcoding using plastid genome sequences (Borisjuk et al. 2014), together with those of *Wa. caudata* and *Wa. gladiata*. However, differentiation of these two species is possible by morphological means, as shown by the pictures here. *Wa. oblonga* is endemic in warmer regions of America like Mexico and Argentina but can be found also frequently in Florida, USA. In contrast, *Wa. repanda* is restricted to a few areas of southern Africa such as Angola and Botswana, in addition to Zimbabwe.

Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ), with input by Klaus J. Appenroth on the text.

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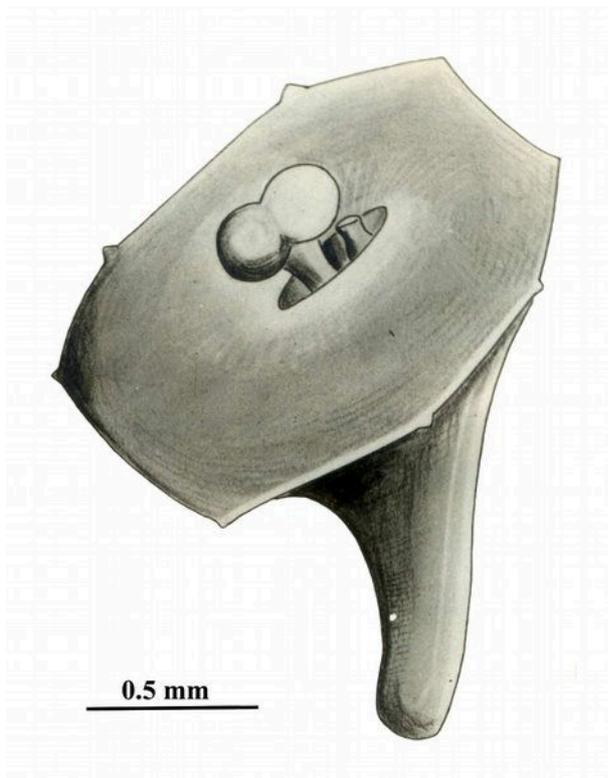
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Information about the ISCDRA: <http://lemnopedia.org/wiki/ISCDRA>

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Science meets art: *Wolffia microscopica* (Griff.) Hartog & Plas



A flowering frond of *Wolffia microscopica* showcasing the male and female floral organs emerging out of the dorsal floral cavity. Drawing by Prof. Dr. Satish C Maheshwari, India.

The upcoming 4th ICDRA in India is dedicated to Prof. S. C. Maheshwari.

Maheshwari, S.C., (Ph.D. Thesis) 1958. The Lemnaceae. A contribution to their biology, morphology and systematic. University of Delhi, Delhi, India.

Letter from the editor

Dear friends of duckweed research and applications,

the **4th International Conference on Duckweed Research and Applications** (ICDRA-2017) is ahead and will start in a few days, 23 – 26 of October 2017 in Kerala, India. This conference is dedicated to Prof. Dr. Satish C. Maheshwari, Rajasthan, India. S. C. Maheshwari belongs to the pioneers of duckweed research. He reported in the journal *Nature* in 1956 about the embryology of *Wolffia microscopica* and in the same journal in 1963 about flowering of this species. Beside many other papers, he co-authored a manuscript about the rediscovery and morphology of *W. microscopica* published in the journal *Flora* in 2015. For our section “Science meets art”, Dr. K. Sowjanya Sree selected one of the drawings from his Ph.D. thesis “The Lemnaceae. A contribution to their biology, morphology and systematic” in 1958. He will be honoured in our ICDRA-2017.

Marvin Edelman, Israel, K. Sowjanya Sree, India and I are going to serve as guest editors for a special issue (Duckweed: Biological Chemistry and Applications) of the journal “Frontiers in Chemistry: Agricultural Biological Chemistry”. Interested scientists may contact one of the guest editors.

Connected with the conference, the election of the next “International Steering Committee on Duckweed Research and Applications” (ISCDRA) is still running until 12nd of October. The voting form is in <https://goo.gl/isaJb2> and the results will be announced during the General Assembly of the ICDRA-2017 in Kerala. During this General Assembly, we will also decide the place of the next ICDRA in 2019.

In the present issue of “Duckweed Forum”, we will have a summary about the progress in Duckweed Molecular Biology. Many readers will be surprised about the huge progress in the last few years. In another report, invasion and disappearance of one species, *Lemna turionifera*, in the central state of Thuringia, Germany will be reported. About the determination of growth rates of duckweed, we had contributions already in previous issues of our Newsletter. However, often authors of scientific publications use something like percentage of increase of biomass (or number of fronds) and call it growth rate. In our chapter “Discussion corner”, we present a comprehensive note on this topic as a contribution to standardizing methods in duckweed research. We open it to our readers to share their opinion and suggestions.

Of course, we have our standard contributions such as the duckweed photos from Eric Lam of the Rutgers Duckweed Stock Cooperative on the cover page, and again the newsletter is appended by the recent literature on Lemnaceae in the chapter “From the Database”.

Best wishes to all of you.

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

Highlights in molecular biology of duckweeds (Lemnaceae)

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In order to use a specific plant in modern plant physiology or biotechnology, several preconditions have to be fulfilled. To make this plant useful for a broad range of researchers, (1.) the whole genome and perhaps also the DNA of plastids and mitochondria must be sequenced and a validated and accurate map must be assembled. Moreover, (2.) genomic transformation and (3.) crossbreeding should be possible and the related protocols should be made available.

In the present article, we would like to inform the readers about the current status of these preconditions for the duckweed family, Lemnaceae.

Whole genome sequencing

Spirodela polyrhiza

After investigating a large number of duckweed species and clones, Wang et al. (2011) confirmed that *Spirodela polyrhiza* has the smallest genome in the duckweed family. This genome has almost exactly the same size as *Arabidopsis thaliana*. Bog et al. (2015) investigated 38 clones of *S. polyrhiza* and measured the genome size as 160 ± 3 Mbp/ 1C. This means that there are hardly any intraspecific differences in the genome size of this species and the same holds true for *S. intermedia* and *Landoltia punctata* (Bog et al., 2015). This is in strong contrast to the large variation in chromosome number ranging between $2n=30$ and $2n=50$ as published by Urbanska-Worytkiewicz (1980). Recent cytogenetic investigations by the group of Ingo Schubert, Gatersleben, Germany demonstrated that all the investigated clones of *S. polyrhiza* have $2n=40$ chromosomes. Interestingly, the genome size of *S. intermedia* was found to be 160 ± 2 Mbp/1C ($n=14$) and that of *L. punctata* to be 421 ± 4 Mbp/1C, suggesting that alteration in chromosome number may be involved in the speciation process (Bog et al., 2015). Whole genome sequencing of *S. polyrhiza* (clone 7498 from North Carolina, USA) was first reported by Wang et al. (2014). Annotation of this first genome draft with 20X coverage revealed that it likely has only 19,623 predicted protein-coding genes, which is 28% less than the dicotyledonous *Arabidopsis thaliana* and 50% less than the monocotyledonous rice genome. Using non-repetitive sequences, BAC positions on chromosomes were investigated with multicolour FISH and the information was used to order the sequence contigs onto 20 chromosome models (Cao et al., 2016). Recently, the genome of *S. polyrhiza* clone 9509 (originally isolated from the outskirts of Jena, Germany) was also sequenced (Michael et al. 2017). Rapid genome-wide physical maps combined with high-coverage short-read sequencing (about 100X coverage) were prepared to resolve the 20 chromosomes of *S. polyrhiza* and to empirically delineate its genome features. This work produced the first genome-wide methylome map for *S. polyrhiza* as well as the first empirically generated small RNA transcriptome that included the siRNA and miRNA components in the genome, in addition to validating the 19 k protein-encoding genes. This comprehensive collection of genomic information enabled the identification of potential centromeric repeats as well as revealed *S. polyrhiza* has the smallest array of ribosomal repeats of any plant tested to date. Currently, efforts between the research groups of Ingo Schubert, Gatersleben,

Germany, Eric Lam, Rutgers University New Jersey, USA and Todd Michael, J. Craig Venter Institute, California, USA promise further validation and improvements in the quality of the *S. polyrhiza* genome assembly using additional cytogenetic studies and ultra-long read nanopore sequencing. A completely resolved *Spirodela* genome will facilitate its reliable use by the community and attract more molecular biologists to the duckweed family.

Other duckweed species

Whole genome sequencing has been reported for *Lemna minor* clone 5500 (van Hoeck et al., 2015). The genome size was reported to be 472 Mbp/1C having $2n=40$ chromosomes. Genome sequencing of this species was carried out in order to make use of the information in RNA-seq analysis on the stress effects of high energy radiation. While this draft version covers 98% of the predicted genome size it has lower contiguity (scaffold length) than the *S. polyrhiza* genome, but we hope that this draft genome and its assembly will be improved in the near future. However, the present version is already of great help in RNA-seq analysis and “genotyping by sequencing” (Bog et al., unpublished results).

Evan Ernst from Cold Spring Harbor Lab, USA in Rob Martienssen lab reported last year the genome sequencing of *L. gibba* clone 7742a and *L. minor* clone 8627 in this newsletter (Ernst, 2016). These results are badly awaited as *L. minor* and *L. gibba* are the species used in most phytotoxicity investigations and most frequently used in duckweed research. Finally, Todd Michael will present an update on his work with Eric Lam on whole genome sequencing and analysis of two *Wolffia australiana* clones during the 4th ICDRA in Kerala, India this year. To the best of our knowledge, until now no one has sequenced any species from the genera *Landoltia* and *Wolffiella*.

Sequencing of plastidic and mitochondrial genomes

The first plastome sequence of *L. minor* (unidentified clone) was already published by Mardanov et al. (2008). Plastidic sequence of *S. polyrhiza* clone 7498 was published by Wang and Messing (2011) and those for mitochondria of the same clone by Wang et al. (2012). Wang and Messing (2011) also reported plastome sequences of two other members of Lemnaceae, *Wolffiella lingulata* 7289 and *Wolffia australiana* 7733. As a consequence, sequences of plastomes from all genera of Lemnaceae are available except for *Landoltia*.

Genetic transformation

Genetic transformation of *L. minor* and *L. gibba* was reported by Yamamoto et al (2001) from the research group of Anne-Marie Stomp, Carolina State University, USA with transgene expression levels reaching up to 7% of soluble protein for a monoclonal antibody (Stomp, 2005). These authors patented the method already some years earlier (Stomp and Rajbhandari, 1998). The efficiency, however, was most probably low as several groups complained that they were not successful in reproducing this method. Marvin Edelman and co-workers, Weizmann Institute of Science, Rehovot, Israel transformed *Landoltia punctata* (*Spirodela oligorrhiza*) and reported finally the stable, transgenic, GFP expression level of 28% of total soluble protein (Edelman et al., 1997; 1998; 2003; Vunsh et al., 2007). More recently, a protocol for agrobacterium-mediated transformation of *L. minor* was reported from the group of R. Martienssen (Canto-Pastor et al., 2015) that promises “efficient transformation”. John W. Cross reported about the “rise and fall of a duckweed biotechnology firm”, Biolex, using genetically transformed duckweed, in the Duckweed Forum (Cross, 2015). He summarized also quite a large number of patents related to the use of transformed duckweeds. The agrobacterium-mediated transformation of *Wolffia arrhiza* reported by the group of Pavel Khvatkov, Moscow, Russia was having an efficiency of 0.2 to 0.4 % in producing stable lines. Experiments with the biolistic method were not successful (Khvatkov et al., 2015).

It should be mentioned here that the group of M. Edelman from Weizmann Institute of Science, Rehovot Israel were successful in producing polyploids of *L. punctata* (*S. oligorrhiza*) and these

authors described interesting changes in physiological properties of these polyploids (Vunsh et al., 2015).

Crossbreeding

Crossbreeding by artificial pollen transfer is another important method in molecular biology, e.g. in trait mapping and genetic studies. The dominant mechanism of propagation in duckweeds is vegetative propagation, as has been known for a long time (Landolt and Kandeler, 1987). However, in many species flowering has been reported (Landolt and Kandeler, 1987; Sree et al., 2015). *Lemna gibba* and *L. aequinoctialis* might be the best investigated systems for flower induction of Lemnaceae (Khurana et al., 1992 and references therein; Pieterse, 2013; Khurana et al., 2014). A closer inspection, however, resulted in the conclusion that all previous researchers investigated exclusively the induction of flowers in *L. gibba* resulting in the formation of flower primordia. Fu et al. (2017) reported recently that seeds formation, after application of salicylic acid, can be enhanced by solidifying the growth medium. Where *L. gibba* clone 7741 showed high frequency of flowering after artificial fertilization, clone 5504 turned out to be male sterile. Using the method of ISSR (Inter-Simple Sequence Repeat) it was demonstrated that intraspecific hybrids were formed, which is important for biotechnology (Fu et al., 2017). However, the same procedure did not work with *L. minor* 7210. In future, we need to extend these investigations to all species of duckweeds.

Acknowledgements

We thank Prof. Dr. Eric Lam, Rutgers University and Dr. Todd P Michael, J. Craig Venter Institute for helpful suggestions and comments on the manuscript.

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Lemna turionifera Landolt – appearance and disappearance

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The species *Lemna turionifera*, one out of 13 species of the genus *Lemna* (Lemnaceae), was first described by Landolt (1975) after he inspected a collection in Montana, Lincoln Co., leg. Wm. M. Hiese 196, 1953, which he integrated in his collection in Zurich, Switzerland under the ID 6753. The main occurrence of this species is in Northern America, i.e. Canada, USA and Mexico. The first report from Germany was in Hamburg (Heckmann, 1984) and Berlin (Scholz, 1995; cited after Landolt 1986). Later, this species was found also on other places in Germany (Wolff and Orschied, 1993). It is assumed that the plant was introduced to Germany by men. Also in 1993, Caspari reported this species for the first time in the central German state, Thuringia. He described several ponds with quite different coverage, from sparsely to 15 % surface coverage, in co-culture with *Lemna minor*.

These ponds close to Weimar, Ehringsdorf are the following given with the local term and the GPS data:

- “Brauereiteich” – 50.957786 N, 11.353431 E
- “Fischerwand” quarry – 50.956285N, 11.347638E
- “Burgholz” – 50.952514N, 11.359611E and 50.953271N, 11.358281E

In 2017, the present authors inspected all ponds described by Caspari (1993). In two ponds only a few fronds were found, which were identified on a morphological basis as *L. minor*. Two other ponds were well covered by duckweeds, which were identified as *L. minor* and *Lemna trisulca*. Two other ponds were dried out. We inspected the ponds in spring and midsummer but in no case, we found the species *L. turionifera*.

Acknowledgement: I thank Dr. Ludwig Ballani for his support.



Duckweed population at "Fischerwand" consisted only of *Lemna minor*.

We conclude in agreement with Wolff and Rabe (1991) that *L. turionifera* can easily colonize new water bodies. This was also confirmed by the fact that Caspari (1993) found this species in all of the described ponds. However, the present observation showed that this does not mean that the species stays thereafter forever. Evidently, the conditions for long-term settlement are different from those required for colonization.

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Discussion Corner

Once more: Growth rates of duckweeds

by Klaus-J. Appenroth, University of Jena, Plant Physiology, Dornburger Str. 159, 07743 Jena, Germany. Email: Klaus.Appenroth@uni-jena.de

The determination of growth rates of duckweed was already described in our newsletter Duckweed Forum 3 (2): 59-62 (2015). However, as unreliable methods were used again and again in the literature, I would like to summarize some important points here. The determination bases on the measurement of the number of fronds (not colonies as the size of colonies can be changed over the experimental period!) at different time points. Instead of frond number, also fresh weight or dry weight can be measured as the parameter.

Growth of a population of individuals can be often described as an exponential increase of the measured parameter. This holds true for bacteria, yeast cells, algae or duckweed fronds. Given that N_0 and N_t are the above mentioned parameter such as frond number, fresh weight or dry weight at time zero and any given time t , the following differential equation can be applied

$$dN/dt = k * t \quad (1)$$

and used after differentiation to result in the basic formula (2):

$$N_t = N_0 * \exp(k * t) \quad (2)$$

The parameter k (often given as per day, d^{-1}) is the relative growth rate (RGR) and can be given as final result of the experiment – of course together with an error. When in an experimental time series parameters were measured at different time points, then the fitting of this equation to these data points is the best way of evaluation. Many computer programs make such fitting easy, e.g. SigmaPlot or SPSS. However, very often only two time points were selected for measurements, e.g. time zero and after 7 days of cultivation. Then equation (2) can be simplified:

$$RGR (d^{-1}) = (\ln N_t - \ln N_0) / (t_7 - t_0) \quad (3)$$

The term RGR is not very demonstrative. Under a RGR of $0.5 d^{-1}$ only expert can imagine something. Therefore two other terms have been derived from equations 2 or 3: doubling time DT and relative (weekly) yield RY. Both parameters were presented and described by Ziegler et al. (2015). Here in short, the doubling time DT is simply the time (often given in days) when frond number, fresh weight or dry weight have doubled relative to the starting point. Short doubling time means fast growth of the population. This can be calculated when the RGR is known:

$$DT (d) = \ln 2 / RGR \quad (4)$$

Alternatively, the relative (weekly) yield RY can be calculated when RGR is known:

$$\ln N_t = \ln N_0 + RGR * (t_7 - t_0) \quad (5).$$

The relative yield RY is the parameter N_t . Ziegler et al. (2015) suggested calculating the relative weekly yield under standardized starting conditions. The meaning of this parameter is the number of fronds (or the amount of fresh weight or dry weight) resulting after one week of cultivation when the experiment was started with one frond (or one g fresh weight or one g dry weight). This parameter is much easier to understand, especially when practical applications are intended. The final result could be e.g. $RY = 224$. This would mean, when you start an experiment with 1 metric ton of fresh weight then after 1 week 224 t are available.

For practical applications, often “growth rates” are given as percent of growth (of fresh weight or dry weight) per area, something like $k = (N_t - N_0) * 100 / N_0 * t$. By the following reasons such a way of calculation should be strictly avoided:

1. This model supposes a linear increase, not an exponential increase. Thus, this “growth rate”

implies a parameter characterizing the properties of a certain population. However, this is not the case. This linear “growth rate” changes from period to period even when growth remains strictly exponential.

2. Real growth rates RGR are always initial growth rates. This means, they are only constant under non-limiting conditions which is possible only over a certain period of time, i.e. at the beginning. After some extended growth either nutrient become limited or plants cover the whole surface and get in touch with each other. R. Kandeler and co-workers (Farber et al. 1989, 1990) showed already that plants respond to this touching by increasing intracellular calcium concentrations, production of ethylene and decrease of growth. This means that over a long period, e.g. growth season, no growth rate can be determined because growth cannot be constant. Assuming a linear increase does not help because the growth rate is changing over the time anyway.

3. Sometimes, authors try to give “growth rates per area”. This is not possible, as growth rates are always related to the starting situation but not to the area.

It is understandable, that duckweed farmers want to characterize the growth of their duckweed under the given cultivation conditions. There are two ways to do this:

The relative growth rate according to equations 2 or 3 can also be calculated in outdoor experiments, at least approximately neglecting the fluctuation of the environmental conditions over the test period. However, this has to be done for an “initial” period to avoid the above mentioned effects and cannot be given per area. In this case, simply the “harvest” should be given after a defined period (like growth season) and per area (e.g. per square meter or ha). This would be clearly defined and is easy to understand.

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From the database

Highlights

Integrated analysis of transcriptome and metabolites reveals an essential role of metabolic flux in starch accumulation under nitrogen starvation in duckweed

Yu, C.J., Zhao, X.W., Qi, G., Bai, Z.T., Wang, Y., Wang, S.M., Ma, Y.B., Liu, Q., Hu, R.B., Zhou, G.K. BIOTECHNOLOGY FOR BIOFUELS 10: Article Number: 167 (2017)

Duckweed is considered a promising source of energy due to its high starch content and rapid growth rate. Starch accumulation in duckweed involves complex processes that depend on the balanced expression of genes controlled by various environmental and endogenous factors. Previous studies showed that nitrogen starvation induces a global stress response and results in the accumulation of starch in duckweed. However, relatively little is known about the mechanisms underlying the regulation of starch accumulation under conditions of nitrogen starvation. In this study, we used next-generation sequencing technology to examine the transcriptome responses of *Lemna aequinoctialis* 6000 at three stages (0, 3, and 7 days) during nitrogen starvation in the presence of exogenously applied sucrose. Overall, 2522, 628, and 1832 differentially expressed unigenes (DEGs) were discovered for the treated and control samples. Clustering and enrichment analysis of DEGs revealed several biological processes occurring under nitrogen starvation. Genes involved in nitrogen metabolism showed the earliest responses to nitrogen starvation, whereas genes involved in carbohydrate biosynthesis were responded subsequently. The expression of genes encoding nitrate reductase, glutamine synthetase, and glutamate synthase was down-regulated under nitrogen starvation. The expression of unigenes encoding enzymes involved in gluconeogenesis was up-regulated, while the majority of unigenes involved in glycolysis were down-regulated. The metabolite results showed that more ADP-Glc was accumulated and lower levels of UDP-Glc were accumulated under nitrogen starvation, the activity of AGPase was significantly increased while the activity of UGPase was dramatically decreased. These changes in metabolite levels under nitrogen starvation are roughly consistent with the gene expression changes in the transcriptome. Based on these results, it can be concluded that the increase of ADP-glucose and starch contents under nitrogen starvation is a consequence of increased output from the gluconeogenesis and TCA pathways, accompanied with the reduction of lipids and pectin biosynthesis. The results provide novel insights into the underlying mechanisms of starch accumulation during nitrogen starvation, which provide a foundation for the improvement of advanced bioethanol production in duckweed.

Flower induction, microscope-aided cross-pollination, and seed production in the duckweed *Lemna gibba* with discovery of a male-sterile clone

Fu, L.L., Huang, M., Han, B.Y., Sun, X.P., Sree, K.S., Appenroth, K.J., Zhang, J.M. SCIENTIFIC REPORTS 7: Article Number: 3047 (2017)

Duckweed species have a great potential to develop into fast-growing crops for water remediation and bioenergy production. Seed production and utilization of hybrid vigour are essential steps in this process. However, even in the extensively-studied duckweed species, *Lemna gibba*, flower primordia were often aborted prior to maturation. Salicylic acid (SA) and agar solidification of the medium promoted flower maturation and resulted in high flowering rates in *L. gibba* 7741 and 5504. Artificial cross-pollination between individuals of *L. gibba* 7741 yielded seeds at high frequencies unlike that in *L. gibba* 5504. In contrast to clone 7741, the anthers of 5504 did not dehisce upon maturation, its artificially released pollen grains had pineapple-like exine with tilted spines. These pollens were not stained by 2,5-diphenylmonotetrazoliumbromide (MTT) and failed to germinate. Therefore, clone

5504 is male sterile and has potential application with respect to hybrid vigour. Moreover, pollination of flowers of 5504 with 7741 pollen grains resulted in intraspecific hybrid seeds, which was confirmed by inter-simple sequence repeat (ISSR) markers. These hybrid seeds germinated at a high frequency, forming new clones.

Biotechnology

Engineering *Brevibacterium flavum* for the production of renewable bioenergy: C4-C5 advanced alcohols

Su, H.F., Lin, J.F., Wang, Y.H., Chen, Q., Wang, G.W., Tan, F.R. BIOTECHNOLOGY AND BIOENGINEERING 114: 1946-1958 (2017)

Biosynthesis of advanced biofuels by engineered non-natural microorganisms has been proposed to be the most promising approach for the replacement of dwindling fossil fuel resources. *Brevibacterium flavum* (Bf) is a model brevibacterium aerobe which lacks basic and applied research that could enable this species to produce biofuels. There are no reports regarding engineering this microorganism to produce advanced alcohols before. Here, for the first time, we developed the bacterium as a novel biosynthetic platform for advanced alcohols production via the mutagenesis and engineering to produce 2-ketoacids derived alcohols. In order to enhance the strain's capability of producing advanced alcohols, we preferentially improved intrinsic metabolism ability of the strain to obtain improved expression host (IEH) via generating mutagenesis libraries by whole cell mutagenesis (WCM). The IEH was determined via screening out the mutant strain with the highest production of branched-chain organic acids (BCOA) using high throughput screening method. Subsequently, a novel vector system for Bf was established, and the corresponding biosynthetic pathway of directing carbon flux into the target advanced alcohols was recruited to make the bacterium possess the capability of producing advanced alcohols and further enhance the production using the IEH. Specifically, we generated bioengineered strains that were able to synthesize up to the highest 5362 and 4976 mg/L isobutanol, 1945 and 1747 mg/L 2-methyl-1-butanol (2MB), and 785.34 and 781 mg/L 3-methyl-1-butanol (3MB) from pure glucose and duckweed substrates, respectively. Our findings confirmed the feasibility and potential of using Bf as a novel biosynthetic platform to generate advanced biofuels with glucose and inexpensive renewable feedstock-duckweed as a fermentation substrate.

Potential of aquatic weeds (*Lemna gibba*, *Lemna minor*, *Pistia stratiotes* and *Eichhornia sp.*) in biofuel production

Gusain, R., Suthar, S. PROCESS SAFETY AND ENVIRONMENTAL PROTECTION 109: 233-241 (2017)

The aim of this study was to assess the biofuel production efficiency of aquatic weeds *Lemna gibba*, *Lemna minor*, *Pistia stratiotes* and *Eichhornia sp.* In order to see the thermo chemical properties of the weed biomass, the proximate, elemental (C, H, N and, O) and, biochemical (carbohydrate, starch and lipids) characteristics of biomass were determined. The lipids from plant biomass was also extracted and then analyzed for preparing the fatty acid profiling (FAME). The results revealed the content of volatile matter, fixed carbon, ash, C, H, N and, O in the ranges of 44.6-59, 18.8-26.3, 18.7-24.9, 32.03-38.02, 4.39-4.87, 2.54-5.31 and, 51.81-59.99%, respectively in dried weed biomass. The Fourier transform infrared spectroscopy (FTIR) analysis suggested the presence of high energy molecules in weed biomass. The content of starch in weed biomass was comparable with other industrial crops being used for bioethanol production. The GC-FID analysis of lipids (FAME analysis) indicated the presence of C16:0, C18:0, C18:1, C18:2 and C18:3 as dominant fatty acids (FAs), which found commonly in biodiesel. The bioethanol production efficiency of weed biomass was also investigated using a microreactor-based trial. Results thus showed a yield of 0.218, 0.197, 0.215 and, 0.189g ethanol in g(-1) dry biomass of *L. minor*, *L. gibba*, *P. stratiotes* and, *Eichhornia sp.*, respectively. Our results conferred that the biomass of aquatic weeds can be utilized as potential feedstock for production of ethanol, butanol, biodiesel, etc. under the clean energy initiatives.

An assessment of duckweed as a potential lignocellulosic feedstock for biogas production

Yadav, D., Barbora, L., Bora, D., Mitra, S., Rangan, L., Mahanta, P. INTERNATIONAL BIODETERIORATION & BIODEGRADATION 119: 253-259 (2017)

Due to the complicated structure of lignocellulosic plant cell wall, their utilization for biogas production via anaerobic digestion has not been widely adopted. Alternative to this is to use aquatic plant materials as feedstock for biogas production. In this context, duckweed, an aquatic plant may prove to be a promising new energy source for bioenergy as well efficient CO₂ sequestration. This study entails a detailed characterization of duckweed to evaluate their potential as an alternate feedstock to cattle dung for biogas production. The duckweed was characterized for volatile matter, moisture content, ash content and carbon, hydrogen, and nitrogen (CHN) content. Property analysis of duckweed was also done by Fourier transform spectroscopy and thermogravimetric analysis. The volatile matter of duckweed was found to be 84.24 +/- 0.2% with a lignin content of 12.2%, which is very encouraging for biogas production. Co-digestion of duckweed (DW) with cattle dung (CD) in varying ratios (DW:CD = 90:10, 75:25, and 50:50 respectively) in batch type anaerobic digesters was performed at 37 degrees C temperature for 55 days. The cumulative biogas production for CD (100%), DW/CD (90:10), (75:25) and (50:50) was found to be 11,620, 305, 11,695, and 12,070 mL, respectively, which indicated that duckweed can be a potential lignocellulosic feedstock when co-digested with cattle dung at an optimum ratio of 1:1. Methane content of the biogas from co-digested feedstock is comparable to the biogas from cattle dung alone.

High flavonoid accompanied with high starch accumulation triggered by nutrient starvation in bioenergy crop duckweed (*Landoltia punctata*)

Tao, X., Fang, Y., Huang, M.J., Xiao, Y., Liu, Y., Ma, X.R., Zhao, H. BMC GENOMICS 18: Article Number: 166 (2017)

As the fastest growing plant, duckweed can thrive on anthropogenic wastewater. The purple-backed duckweed, *Landoltia punctata*, is rich in starch and flavonoids. However, the molecular biological basis of high flavonoid and low lignin content remains largely unknown, as does the best method to combine nutrients removed from sewage and the utilization value improvement of duckweed biomass. A combined omics study was performed to investigate the biosynthesis of flavonoid and the metabolic flux changes in *L. punctata* grown in different culture medium. Phenylalanine metabolism related transcripts were identified and carefully analyzed. Expression quantification results showed that most of the flavonoid biosynthetic transcripts were relatively highly expressed, while most lignin-related transcripts were poorly expressed or failed to be detected by iTRAQ based proteomic analyses. This explains why duckweed has a much lower lignin percentage and higher flavonoid content than most other plants. Growing in distilled water, expression of most flavonoid-related transcripts were increased, while most were decreased in uniconazole treated *L. punctata* (1/6 x Hoagland + 800 mg center dot L⁻¹ uniconazole). When *L. punctata* was cultivated in full nutrient medium (1/6 x Hoagland), more than half of these transcripts were increased, however others were suppressed. Metabolome results showed that a total of 20 flavonoid compounds were separated by HPLC in *L. punctata* grown in uniconazole and full nutrient medium. The quantities of all 20 compounds were decreased by uniconazole, while 11 were increased and 6 decreased when grown in full nutrient medium. Nutrient starvation resulted in an obvious purple accumulation on the underside of each frond. The high flavonoid and low lignin content of *L. punctata* appears to be predominantly caused by the flavonoid-directed metabolic flux. Nutrient starvation is the best option to obtain high starch and flavonoid accumulation simultaneously in a short time for biofuels fermentation and natural products isolation.

Feed and Food

Duckweeds as human food

Hamalton, T., Prabha, A.C.S., Tekam, M.K., Nayak, P.P., Mahadevaswamy, H.M., Udham, P.K., Subashini, V., Chandra, G.S., Iqbal, S. CURRENT SCIENCE 112: 675-675 (2017)

No abstract available.

Phytoremediation

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

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

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

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

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

The fate of five benzotriazoles (1H-benzotriazole, BTR; 4-methyl-1H-benzotriazole, 4TTR; 5-methyl-1H-benzotriazole, 5TTR; xylotriazole, XTR and 5-chlorobenzotriazole, CBTR) was studied in batch and continuous-flow *Lemna minor* systems and the role of different mechanisms on their removal was evaluated. Single and joint toxicity experiments were initially conducted using the Organization for Economic Co-operation and Development (OECD) protocol 221 and no inhibition on specific growth rate of *Lemna minor* was observed for concentrations up to 200 $\mu\text{g L}^{-1}$. All tested substances were significantly removed in batch experiments with *Lemna minor*. Excepting 4TTR, full elimination of CBTR, XTR, 5TTR and BTR was observed up to the end of these experiments (36 d), while the half-life values ranged between 1.6 \pm 0.3 d (CBTR) and 25 \pm 3.6 d (4-TTR). Calculation of kinetic constants for hydrolysis, photodegradation, and plant uptake revealed that for all BTRs the kinetic constants of plant uptake were by far higher comparing to those of the other mechanisms, reaching 0.394 \pm 0.161 d⁻¹ for CBTR. The operation of a continuous-flow *Lemna minor* system consisted

of three mini ponds and a total hydraulic residence time of 8.3 d showed sufficient removal for most target substances, ranging between 26% (4TTR) and 72% (CBTR). Application of a model for describing micropollutants removal in the examined system showed that plant uptake was the major mechanism governing BTRs removal in Lemna minor systems.

Antioxidant response in duckweed after exposure to secondary effluent from municipal wastewater treatment plant, Elazig, Turkey

Tatar, S.Y., Obek, E., Yildirim, N.C. BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 99: 399-404 (2017)

The aim of this study is to evaluate the effects of the effluent of Elazig Municipality Wastewater Treatment Plant on the oxidative defense capacity of aquatic plants (*Lemna minor* L. and *Lemna gibba* L.). For this purpose, malondialdehyde (MDA), glutathione (GSH), oxidized glutathione (GSSG), vitamin A (retinol), vitamin E (alpha-tocopherol), and vitamin C (Ascorbic acid) levels were determined by the HPLC (high performance liquid chromatography) in the control groups and the groups adapting to reactors fed with discharge water. The depletion of vitamins (A, E, and C), decrease of GSH/GSSG ratio, and increase of MDA that reflect a precarious state of the cell in *L. minor* L. and *L. gibba* L. were observed after exposure to wastewater. It can be suggested that the selected biomarkers are useful in understanding the biochemical mechanisms of the secondary effluents from wastewater treatment plant in *L. minor* L. and *L. gibba* L. as early warning indicators.

Enantioselective accumulation, metabolism and phytoremediation of lactofen by aquatic macrophyte *Lemna minor*

Wang, F., Yi, X.T., Qu, H., Chen, L., Liu, D.H., Wang, P., Zhou, Z.Q. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 143: 186-192 (2017)

Pesticides are frequently detected in water bodies due to the agricultural application, which may pose impacts on aquatic organisms. The enantioselective bioaccumulation and metabolism of the herbicide lactofen in aquatic floating macrophyte *Lemna minor* (*L. minor*) were studied and the potential *L. minor* phytoremediation was investigated. Ultra-high performance liquid chromatography - tandem mass spectrometry (UHPLC-MS-MS) analysis for lactofen and its two known metabolites in *L. minor* was performed. The initial concentrations of racemic lactofen, R-lactofen and S-lactofen were all 30 $\mu\text{g L}^{-1}$ in the growth solution. The distribution of lactofen and its metabolites in growth solution and *L. minor* was determined throughout a 5-d laboratory trial. It was observed that S-lactofen was preferentially taken up and metabolized in *L. minor*. After rac-lactofen exposure, the accumulation amount of S-lactofen was approximately 3-fold more than that of R-lactofen in *L. minor* and the metabolism rate of S-lactofen ($T_{1/2} = 0.92$ d) was significantly faster than R-lactofen ($T_{1/2} = 1.55$ d). *L. minor* could only slightly accelerate the metabolism and removal of lactofen in the growth solution. As for the metabolites, desethyl lactofen was found to be the major metabolite in *L. minor* and the growth solution, whereas the metabolite acifluorfen was undetectable. No interconversion of the two enantiomers was observed after individual enantiomer exposure, indicating they were configurationally stable. The findings of this work represented that the accumulation and metabolism of lactofen in *L. minor* were enantioselective, and *L. minor* had limited capacity for the removal of lactofen and its metabolite in water.

Abundance, activity and community structure of denitrifiers in drainage ditches in relation to sediment characteristics, vegetation and land-use

Veraart, A.J., Dimitrov, M.R., Schrier-Uijl, A.P., Smidt, H., de Klein, J.J.M. ECOSYSTEMS 20: 928-943 (2017)

Drainage ditches are ubiquitous yet understudied features of the agricultural landscape. Nitrogen pollution disrupts the nutrient balance of drainage ditch ecosystems, as well as the waterbodies in which they drain. Denitrification can help ameliorate the impact of N-fertilization by converting reactive nitrogen into dinitrogen gas. However, factors affecting denitrification in drainage ditches

are still poorly understood. In this study, we tested how within-ditch and regional environmental conditions affect denitrifier activity, abundance, and community structure, to understand controls on denitrification at multiple scales. To this end, we quantified in situ denitrification rates and denitrifier abundance in 13 drainage ditches characterized by different types of sediment, vegetation and land-use. We determined how denitrification rates relate to denitrifier abundance and community structure, using the presence of *nirS*, *nirK* and *nosZ* genes as a proxy. Denitrification rates varied widely between the ditches, ranging from 0.006 to 24 mmol N m⁻² h⁻¹. Ditches covered by duckweed, which contained high nitrate concentrations and had fine, sandy sediments, were denitrification hotspots. We found highest rates in ditches next to arable land, followed by those in grasslands; lowest rates were observed in peatlands and nature reserves. Denitrification correlated to nitrate concentrations, but not to *nirK*, *nirS* and *nosZ* abundance, whereas denitrifier-gene abundance correlated to organic matter content of the sediment, but not to nitrate concentrations. Our results show a mismatch in denitrification regulators at its different organizational scales. Denitrifier abundance is mostly regulated at within-ditch scales, whereas N-loads, regulated by landscape factors, are most important determinants of instantaneous denitrification rates.

Can bacterial biofiltration be replaced by autotrophic organisms in recirculating fresh water aquaculture?

Ojanen, S., Tyystjarvi, E., Holmberg, H., Kouhia, M., Ahtila, P. AQUACULTURE INTERNATIONAL 25: 1427-1440 (2017)

In recirculating aquaculture, a bacterial biofilter is applied to convert ammonium, excreted by the fish, to the non-toxic nitrate. Unfortunately, nitrifying bacteria produce off-flavor compounds that lower fish quality. We investigated, by calculations and estimations, possibilities to replace the biofilter by autotrophic organisms that incorporate ammonium in biomass, consume other mineral nutrients and produce marketable biomass and oxygen. The capacity of microalgae, macroalgae, duckweed, strawberry, and tomato to assimilate ammonium was calculated, using data from an existing Finnish fresh water fish farm. Microalgae were found to be the most effective for ammonium removal, and they would be able to consume the ammonium produced by a fish farm if the algae were grown in a facility with approximately twice the area of the fish farm itself. Macroalgae and duckweed appeared to be the second best option for ammonium removal, and strawberry and tomato were predicted to have a somewhat smaller capacity for ammonium removal. Due to low ammonium content, microalgae cannot be cultivated in the recirculating water, but rather the nutrients should be allowed to diffuse through a semipermeable membrane to microalgae.

Comparison of experimental ponds for the treatment of dye wastewater under controlled and semi-natural conditions

Yaseen, D.A., Scholz, M. ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 16031-16040 (2017)

This study compares the performance of simulated shallow ponds vegetated with *Lemna minor* L. under controlled and semi-natural conditions for the treatment of simulated wastewater containing textile dyes. The objectives were to assess the water quality outflow parameters, the potential of *L. minor* concerning the removal of chemical oxygen demand (COD) and four azo dyes (Acid blue 113, reactive blue 198, Direct Orange 46 and Basic Red 46) and the plants' growth rate. Findings show that all mean outflow values of COD, total dissolved solids (TDS) and electrical conductivity (EC) were significantly ($p < 0.05$) lower within the outdoor compared to the indoor experiment except the dissolved oxygen (DO). The COD removal was low for both experiments. The outflow TDS values were acceptable for all ponds. The pond systems were able to reduce only BR46 significantly ($p < 0.05$) for the tested boundary conditions. Removals under laboratory conditions were better than those for semi-natural environments, indicating the suitability of operating the pond system as a polishing step in warmer regions. The mean outflow values of zinc and copper were below the thresholds set for drinking and irrigation waters and acceptable for *L. minor*. The dyes inhibited the

growth of the *L. minor*.

Ecology of duckweed ponds used for nutrient recovery from wastewater

Teles, C.C., Mohedano, R.A., Tonon, G., Belli, P., Costa, R.H.R. WATER SCIENCE AND TECHNOLOGY 75: 2926-2934 (2017)

The microorganism community that grows under duckweed shelter can play an important role on treatment processes. Therefore, the present study aimed to assess the zooplankton dynamic and microbial community in duckweed ponds (DPs) applied for domestic wastewater treatment under open field conditions. A pilot system comprised of two DPs in series (DP1 and DP2), with 10 m² each, received domestic wastewater through a flow rate of 200 L.day⁻¹. Thus, the system was monitored during 314 days through samples collected and analysed weekly. Also, the zooplankton organisms were identified and quantified. DNA sequencing was performed in order to identify the bacterial populations. The findings showed a high efficiency of nutrient removal with 93% and 91% of total phosphorus and total nitrogen, respectively. A high density of microcrustaceans was observed in DP1 reaching 4,700 org.100 mL⁻¹ and rotifers (over than 32,000 org.100 mL⁻¹) in DP2, that could be related to the low suspended solids concentration (< 30 mg . L⁻¹) and turbidity (< 10 NTU). The bacterial community showed a strong heterogeneity between samples collected along the seasons. Through these findings, it is possible to realise that the understanding of ecology could help to enhance the operation and designs of DPs.

Survival, reproduction, growth, and parasite resistance of aquatic organisms exposed on-site to wastewater treated by advanced treatment processes

Schluter-Vorberg, L., Knopp, G., Cornel, P., Ternes, T., Coors, A. AQUATIC TOXICOLOGY 186: 171-179 (2017)

Advanced wastewater treatment technologies are generally known to be an effective tool for reducing micropollutant discharge into the aquatic environment. Nevertheless, some processes such as ozonation result in stable transformation products with often unknown toxicity. In the present study, whole effluents originating from nine different steps of advanced treatment combinations were compared for their aquatic toxicity. Assessed endpoints were survival, growth and reproduction of *Lumbriculus variegatus*, *Daphnia magna* and *Lemna minor* chronically exposed in on-site flow-through tests based on standard guidelines. The treatment combinations were activated sludge treatment followed by ozonation with subsequent filtration by granular activated carbon or biofilters and membrane bioreactor treatment of raw wastewater followed by ozonation. Additionally, the impact of treated wastewater on the immune response of invertebrates was investigated by challenging *D. magna* with a bacterial endoparasite. Conventionally treated wastewater reduced reproduction of *L. variegatus* by up to 46%, but did not affect *D. magna* and *L. minor* with regard to survival, growth, reproduction and parasite resistance. Instead, parasite susceptibility was significantly reduced in *D. magna* exposed to conventionally treated as well as ozonated wastewater in comparison to *D. magna* exposed to the medium control. None of the three test organisms provided clear evidence that wastewater ozonation leads to increased aquatic toxicity. Rather than to the presence of toxic transformation products, the affected performance of *L. variegatus* could be linked to elevated concentrations of ammonium and nitrite that likely resulted from treatment failures.

Effectiveness of vegetated drainage ditches for domestic sewage effluent mitigation

Kumwimba, M.N., Zhu, B. BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 98: 682-689 (2017)

Plant species have an important role in eco-ditches; however, the Michaelis-Menten kinetic parameters of nutrient uptake, growth rate and purification efficiency of ditch plants and their

influences on domestic sewage treatment efficiency are still unclear. Growth rates of all nine species, but especially *Lemna gibba*, *Cladophora* and *Myriophyllum verticillatum* were best in undiluted domestic sewage as opposed to a mixture of domestic sewage. Performance of species to accumulate nutrients was not only species-specific, but was also affected by both sewage treatments. Removal efficiency of nutrients was dependent on both plant species and treatment. Uptake kinetic parameters were significantly affected by both nutrient form and plant species. The maximum uptake rate (V_{max}) of NH_4-N was higher than NO_3-N . Similarly, K_m values for NH_4-N were greater than NO_3-N . These results could be used to identify plants for sewage treatment efficiency and enhance water quality in eco-ditch treatment systems.

Response of duckweed to lead exposure: phytomining, bioindicators and bioremediation

Hegazy, A.K., Emam, M.H., Lovett-Doust, L., Azab, E., El-Khatib, A.A. DESALINATION AND WATER TREATMENT 70: 227-234 (2017)

The ability of aquatic macrophytes to bioaccumulate toxic metals relative to the concentrations of these metals in wastewater has led to their use as phytoremediators. Lead (Pb) is among the most serious environmental contaminants. This study assesses the gibbous duckweed (*Lemna gibba* L.) as a bioaccumulator and bioindicator of Pb pollution. The plant recovery from a 12-d exposure period in terms of re-releases of Pb from its tissues, and recovery of pigmentation was monitored. Duckweed was exposed to Pb-contaminated water by adding $PbCO_3$ at concentrations from 10 to 100 mg/L. At 2-d intervals, bioaccumulation, contaminant removal efficiency, pigment content, and bleaching were assessed. The efficiency of Pb removal after 12 d reached nearly 50% at the lowest Pb treatment (10 mg/L), but decreased at higher levels of Pb up to 100 mg/L. The highest bioconcentration factors (BCF) were achieved at low Pb treatment of 10 mg/L, which increased from nearly 200 mg/L after 2 d, to 943 mg/L after 12 d of exposure. Recovery from bleaching was around 50% for all photosynthetic pigments in plants exposed to 10-40 mg/L concentrations of Pb. The response of duckweed to Pb treatment and recovery from stress suggest its possible use as biosensor or biomonitor of Pb pollution, considering that active uptake, rather than low concentration gradient, is driving the absorption of Pb from the water medium.

The uses of duckweed in relation to water remediation

Ziegler, P., Sree, K.S., Appenroth, K.J. DESALINATION AND WATER TREATMENT 63: 327-342 (2017)

Duckweeds are small, simply structured floating plants that grow on surface waters. They grow rapidly and are easy to cultivate, harvest, process and analyze, which makes them useful in many ways. Duckweeds are of great value in illustrating the physiological effects of toxic water contaminants on plants and serving to indicate the presence and environmental risk of such toxins. The pronounced capacity of duckweeds to assimilate aqueous nutrients and to take up and mediate the removal of a variety of toxic substances from aqueous solution constitutes the potential of these organisms for wastewater - remediation. The biomass yielded by duckweed growth - particularly on nutrient-rich wastewater has a high nutritional value and is well suited for biofuel production, as well as being useful for fertilization, biosorption and soil and water amendment. Duckweeds thus have the potential for integrating a significant contribution to meeting food, feed and energy demands traditionally supplied by terrestrial crop plants and fossil fuels with the remediation of polluted waters. Duckweed growth can also be used to directly generate bioelectricity, and the success of genetically transforming duckweed plants opens the road to biomanufacturing with these organisms, both of which are in principle compatible with wastewater remediation. However, neither wastewater remediation by duckweeds nor the exploitation of the multiple potential benefits of wastewater-grown duckweed biomass has yet been widely implemented. The present review underlines the potential of duckweeds for combining resource management with water remediation, while examining the difficulties encountered in the realization of this potential.

Phytotoxicity

Ecotoxicity of nanosized magnetite to crustacean *Daphnia magna* and duckweed *Lemna minor*

Blinova, I., Kanarbik, L., Irha, N., Kahru, A. HYDROBIOLOGIA 798: 141-149 (2017)

Along with the development of nanotechnology, an increase in production and application of nanosized magnetite (Fe₃O₄) is expected. Though magnetite is considered relatively safe, information concerning potential hazards of synthetic magnetite nanoparticles with unique physico-chemical characteristics to aquatic organisms is still limited. In this study, we evaluated the toxicity of nanosized (27.2 +/- A 9.8 nm) and bulk (144.2 +/- A 67.7 nm) magnetite particles to different life stages of the aquatic crustacean *Daphnia magna*. In addition, phytotoxicity of the magnetite was evaluated using duckweed *Lemna minor*. The study did not reveal any statistically significant differences between the biological effects of nanosized and bulk magnetite particles. Both forms of magnetite induced very low toxicity (EC₅₀ > 100 ppm) to *D. magna* and *L. minor* in the standard acute assays. However, it was demonstrated that at acutely subtoxic magnetite concentrations (10 and 100 ppm), the number of neonates hatched from *D. magna* ephippia was decreased. Moreover, short-term (48 h) exposure of neonate daphnids to these concentrations may significantly affect the long-term survival and reproductive potential of daphnids. These results indicate that substantial contamination of aquatic ecosystems by magnetite may disrupt the stability of cladoceran populations.

Mercury induced oxidative stress, DNA damage, and activation of antioxidative system and Hsp70 induction in duckweed (*Lemna minor*)

Zhang, T.T., Lu, Q.Q., Su, C.L., Yang, Y.R., Hu, D., Xu, Q.S. ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 143: 46-56 (2017)

Mercury uptake and its effects on physiology, biochemistry and genomic stability were investigated in *Lemna minor* after 2 and 6 d of exposure to 0-30 µM Hg. The accumulation of Hg increased in a concentration- and duration-dependent manner, and was positively correlated with the leaf damage. Oxidative stress after Hg exposure was evidenced in *L. minor* by a significant decrease in photosynthetic pigments, an increase in malondialdehyde and lipoxygenase activities (total enzyme activity and isoenzymes activity). Fronds of *L. minor* exposed to Hg showed an induction of peroxidase, catalase, and ascorbate peroxidase activities (total enzyme activity and some isoenzymes activities). Exposure of *L. minor* to Hg reduced the activity (total enzyme activity and some isoenzymes activities) of glutathione reductase, and superoxide dismutase. Exposure to Hg produced a transient increase in the content of glutathione and ascorbic acid. The content of dehydroascorbate and oxidized glutathione in *L. minor* were high during the entire exposure period. Exposure of *L. minor* to Hg also caused the accumulation of proline and soluble sugars. The amplification of new bands and the absence of normal DNA amplicons in treated plants in the random amplified polymorphic DNA (RAPD) profile indicated that genomic template stability (GTS) was affected by Hg treatment. The accumulation of Hsp70 indicated the occurrence of a heat shock response at all Hg concentrations. These results suggest that *L. minor* plants were able to cope with Hg toxicity through the activation of various mechanisms involving enzymatic and non-enzymatic antioxidants, up regulation of proline, and induction of Hsp70.

The biological responses and metal phytoaccumulation of duckweed *Spirodela polyrhiza* to manganese and chromium

Liu, Y., Sanguanphun, T., Yuan, W.Q., Cheng, J.J., Meetam, M. ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 19104-19113 (2017)

The phytoaccumulation ability of duckweed *Spirodela polyrhiza* on manganese (Mn) and chromium (Cr) was assessed by exposing the plant to various concentrations of single or dual metals (5-70 mg L⁻¹ Mn, 2-12 mg L⁻¹ Cr(VI)) under laboratory conditions. The results showed that *S. polyrhiza* can

tolerate Mn at high concentrations of up to 70 mg L⁻¹, and its growth rate was barely affected by Mn. The effects of Cr on *S. polyrhiza* growth were dose-dependent, and the growth was completely inhibited in the presence of 12 mg L⁻¹ Cr. Analysis of metal content in the plant biomass revealed a high accumulation of Mn (up to 15.75 mg per g of duckweed dry weight). The Cr bioaccumulation (from below detection limit to 2.85 mg Cr (11.84 mg Cr₂₀₇₂-) per g of duckweed dry weight) increased with cultivation time and metal concentration in the medium. Further study with the concurrence of Mn and Cr showed increased toxicity to plant growth and photosynthesis. The metal accumulations in the dual metal treatments were also significantly decreased as compared to the single metal treatments. Nevertheless, the phytoaccumulation of these two metals in *S. polyrhiza* in the dual metal treatments were still comparable to or higher than in previous reports. Thus, it was concluded that duckweed *S. polyrhiza* has the potential to be used as a phytoremediator in aquatic environments for Mn and Cr removal.

Lead and copper adsorption behaviour by *Lemna gibba*: kinetic and equilibrium studies

Carreno-De Leon, M.D., Flores-Alamo, N., Solache-Rios, M.J., De la Rosa-Gomez, I., Diaz-Campos, G. CLEAN-SOIL AIR WATER 45: Article Number: 1600357 (2017)

Copper and lead were found in sediments of Chimaliapan Lake in the State of Mexico; these elements may come from local tanneries and other industries. The sorption behaviour of lead and copper by *Lemna gibba* sampled from the same lake was determined. The results showed that the removal capacity for lead (about 98.9%) was higher than for copper (60%). The maximum adsorption capacities were at pH 4 and highest for Pb(II). Adsorption kinetics showed that the experimental data fitted the pseudo-first and -second order models ($R^2 = 0.99$). Equilibrium data of Pb(II) fitted best to the Langmuir model and Cu(II) data to the Langmuir and Freundlich models.

Surface coating-modulated toxic responses to silver nanoparticles in *Wolffia globosa*

Zou, X.Y., Li, P.H., Lou, J., Zhang, H.W. AQUATIC TOXICOLOGY 189: 150-158 (2017)

With the omnipresence of silver nanoparticles (AgNPs) in our daily consumer products, their release has raised serious concerns. However, the biochemical mechanisms by which plants counteract the toxicity of nanoparticles are largely unknown. This study investigated the exposure of aquatic *Wolffia globosa* to ATP-nAg (AgNPs coated with adenosine triphosphate), cit-nAg (AgNPs coated with citrate), and Ag⁺. Hill reaction activity was basically lost in *W. globosa* treated with 10 mg/L ATP-nAg and Ag⁺, while the activity was still maintained at 38.7%-38.9% of the respective controls at 10 mg/L cit-nAg. The reduction of amounts of chlorophyll and soluble protein were shown in response to the Ag stresses. This was accompanied by the accumulation of sugar in *W. globosa* treated with cit-nAg. By contrast, the depletion of sugar was recorded after 10 mg/L ATP-nAg and Ag⁺ treatments. The superoxide dismutase and peroxidase activities were significantly increased after exposure to 10 mg/L ATP-nAg and Ag⁺, which did not occur in *W. globosa* treated with cit-nAg. The ratio between NADPH/NADP(+) was higher after cit-nAg and Ag⁺ stresses than the respective controls. The accumulation of Ag was found to increase in a concentration-dependent manner. Ag⁺ and ATP-nAg inhibited the uptake of P and K, and promoted the uptake of Fe and Cu. In contrast, cit-nAg only promoted the uptake of Cu. Our results implied that surface coating induced different physiological responses of *W. globosa* to AgNPs. Based on above results, we speculated that after exposure to cit-nAg, citrate possibly could serve as the substrate for the tricarboxylic acid cycle and accumulated sugar may promote pentose phosphate pathways. For ATP-nAg treatments, ATP would act as an exogenous energy source of plant metabolisms. Our findings demonstrate that surface coating regulates the physiological responses of plants to AgNPs through distinct mechanisms.

Toxicological implications of selenium nanoparticles with different coatings along with Se⁴⁺ on *Lemna minor*

Tarrahi, R., Khataee, A., Movafeghi, A., Rezanejad, F., Gohari, G. CHEMOSPHERE 181: 655-665 (2017)

Nanoparticles have potential high risks for living organisms in the environment due to their specific qualities and their easy access. In the present study, selenium nanoparticles (Se NPs) with two different coatings (L-cysteine and tannic acid) were synthesized. The characteristics of particles were analyzed using XRD, FT-IR and SEM. The impact of the nanoparticles besides Se⁴⁺, on the aquatic higher plant *Lemna minor* was evaluated and compared. Entrance of L-cysteine and tannic acid capped Se NPs in the roots of *Lemna minor* was proved by TEM and fluorescence microscopy. Adverse effects of mentioned NPs and differences of these effects from those by sodium selenite as the ionic form were assessed by a range of biophysicochemical tests. Altogether, the results asserted that *Lemna minor* was notably poisoned by both capped Se NPs and Se⁴⁺. Thus, growth and photosynthetic pigments were decreased while lipid peroxidation along with total phenol and flavonoid contents were raised. Eventually some changes in enzymatic activities were presented. To sum up the consequences, it can be concluded that all changes occurred due to the plant defense system especially in order to remove reactive oxygen species (ROS) and possible phytotoxicity originated by L-cysteine and tannic acid capped Se NPs in addition to Se⁴⁺. The influence of tannic acid capped Se NPs after sodium selenite is stronger by the means of antioxidant enzymes activity in comparison with L-cysteine capped Se NPs.

Citric acid enhanced the antioxidant defense system and chromium uptake by *Lemna minor* L. grown in hydroponics under Cr stress

Sallah-Ud-Din, R., Farid, M., Saeed, R., Ali, S., Rizwan, M., Tauqeer, H.M., Bukhari, S.A.H. ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 17669-17678 (2017)

Phytoextraction is a cost-effective and eco-friendly technique for the removal of pollutants, mainly heavy metal(oids) especially from polluted water and metal-contaminated soils. The phytoextraction of heavy metals is, in general, limited due to the low availability of heavy metals in the growth medium. Organic chelators can help to improve the phytoextraction by increasing metal mobility and solubility in the growth medium. The present research was carried out to examine the possibility of citric acid (CA) in improving chromium (Cr) phytoextraction by *Lemna minor* (duckweed). For this purpose, healthy plants were collected from nearby marsh and grown in hydroponics under controlled conditions. Initial metal contents of both marsh water and plant were measured along with physico-chemical properties of the marsh water. Different concentrations of Cr and CA were applied in the hydroponics in different combinations after defined intervals. Continuous aeration was supplied and pH maintained at 6.5 +/- 0.1. Results showed that increasing concentration of Cr significantly decreased the plant biomass, photosynthetic pigments, leaf area, and antioxidant enzyme activities (like catalase, ascorbate peroxidase, superoxide dismutase, peroxidase). Furthermore, Cr stress increased the Cr concentrations, electrolyte leakage, hydrogen peroxide, and malondialdehyde contents in plants. The addition of CA alleviated the Cr-induced toxicity in plants and further enhanced the Cr uptake and its accumulation in *L. minor*. The addition of CA enhanced the Cr concentration in *L. minor* by 6.10, 26.5, 20.5, and 20.2% at 0, 10, 100, and 200 μ M Cr treatments, respectively, compared to the respective Cr treatments without CA. Overall, the results of the present study showed that CA addition may enhance the Cr accumulation and tolerance in *L. minor* by enhancing the plant growth and activities of antioxidant enzymes.

Particle size and concentration dependent ecotoxicity of nano- and microscale TiO₂ -Comparative study by different aquatic test organisms of different trophic levels

Fekete-Kertesz, I., Pizsman, D., Molnar, M. WATER AIR AND SOIL POLLUTION 228: Article Number: 245 (2017)

A comprehensive ecotoxicity assessment of three different nanosized TiO₂ (with 16, 36 and 89 nm particle diameter) and one microscale TiO₂ suspension (with 3264 nm particle diameter) was

carried out with a special emphasis on the relation between product characteristics and toxic effect. The applied test battery included the combination of modified standardized tests (Aliivibrio fischeri bioluminescence inhibition test, Lemna minor growth inhibition test), and nonstandardized bioassays with unconventional physiological endpoints (Tetrahymena pyriformis phagocytic activity, the Daphnia magna heartbeat rate). Based on the lowest significant effect values, the tested aquatic organisms were the most sensitive to the microscale TiO₂ suspension (with 3264 nm particle size). Although the three nanoscale TiO₂ particles were aggregated in the A. fischeri and the L. minor growth media, significant inhibition rates were experienced at 0.1 and at 1.g L⁻¹ concentration of nTiO₂ suspensions with 16 and 36 nm primary particle size, respectively. Larger aggregates may have also high impact on biological organisms. In case of the D. magna heartbeat rate test rapid agglomeration was avoided, but lower responses were found compared to other investigated systems. The short term T. pyriformis phagocytic activity test demonstrated outstanding sensitivity; three TiO₂ suspensions were significantly toxic even at 0.1.g L⁻¹. The consequences of our study clearly indicated that nanoscale TiO₂ may have an impact on the aquatic ecosystem which is strongly influenced by aggregation. The effect of exposure duration and concentration as contributing factors in nanotitanium dioxide mediated toxicity was also demonstrated.

Study on mechanism of arsenic tolerance in duckweeds from lead-zinc mine by synchrotron radiation X-ray fluorescence and X-ray absorption near edge structure spectrometry

Chu, B.B., Luo, L.Q., Ma, Y.H. CHINESE JOURNAL OF ANALYTICAL CHEMISTRY 45: 668-673 (2017)

Aquatic plant duckweed can enrich high concentration of arsenic, it is thus used as the representative of phytofiltration. The mechanism of arsenic tolerance in duckweeds has received much concern. In this study, synchrotron radiation X-ray fluorescence (SRXRF) and X-ray absorption near edge structure (XANES) techniques were used to study the micro-distribution and speciation of arsenic in natural As. rich duckweed from lead. zinc mine. Two monolithic duckweeds, FP1 and FP2, were analyzed by micro SRXRF, setting single point scan time and spot size were 5 s, 70 μm x 80 μm and 2 s, 100 μm x 100 μm respectively. Six points of FP2 were selected and analyzed by micro XANES in energy range of 11. 81-11. 96 keV. Pressed. pellet duckweed was analyzed by bulk XANES in energy range of 11. 67-12. 27 keV. The result showed that As. was the major speciation of duckweed from bulk XANES and micro. XANES data. SRXRF micro analysis showed that arsenic had significant vein distribution in duckweed, and was not spread into the photosynthetic mesophyll within certain concentration, which may reduce the leaf toxicity triggered by arsenic. This vein distribution may play a role in arsenic tolerance in duckweed.

Simultaneous determination of multiclass emerging contaminants in aquatic plants by ultrasound-assisted matrix solid-phase dispersion and GC-MS

Aznar, R., Albero, B., Sanchez-Brunete, C., Miguel, E., Martin-Girela, I., Tadeo, J.L. ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 24: 7911-7920 (2017)

A multiresidue method was developed for the simultaneous determination of 31 emerging contaminants (pharmaceutical compounds, hormones, personal care products, biocides, and flame retardants) in aquatic plants. Analytes were extracted by ultrasound-assisted matrix solid-phase dispersion (UA-MSPD) and determined by gas chromatography-mass spectrometry after silylation. The method was validated for different aquatic plants (Typha angustifolia, Arundo donax, and Lemna minor) and a semiaquatic cultivated plant (Oryza sativa) with good recoveries at concentrations of 100 and 25 ng g⁻¹ wet weight, ranging from 70 to 120 %, and low method detection limits (0.3 to 2.2 ng g⁻¹ wet weight). A significant difference of the chromatographic response was observed for some compounds in neat solvent versus matrix extracts, and therefore, quantification was carried out using matrix-matched standards in order to overcome this matrix effect. Aquatic plants taken from rivers located at three Spanish regions were analyzed, and the compounds detected were parabens, bisphenol A, benzophenone-3, cyfluthrin, and cypermethrin. The levels found ranged from

6 to 25 ng g(-1) wet weight except for cypermethrin that was detected at 235 ng g(-1) wet weight in *O. sativa* samples.

Silver nanoparticles induced reactive oxygen species via photosynthetic energy transport imbalance in an aquatic plant

Jiang, H.S., Yin, L.Y., Ren, N.N., Zhao, S.T., Li, Z., Zhi, Y.W., Shao, H., Li, W., Gontero, B. NANOTOXICOLOGY 11: 157-167 (2017)

The rapid growth in silver nanoparticles (AgNPs) commercialization has increased environmental exposure, including aquatic ecosystem. It has been reported that the AgNPs have damaging effects on photosynthesis and induce oxidative stress, but the toxic mechanism of AgNPs is still a matter of debate. In the present study, on the model aquatic higher plant *Spirodela polyrhiza*, we found that AgNPs affect photosynthesis and significantly inhibit Photosystem II (PSII) maximum quantum yield (F-v/F-m) and effective quantum yield (ϕ (PSII)). The changes of non-photochemical fluorescence quenching (NPQ), light-induced non-photochemical fluorescence quenching [Y(NPQ)] and non-light-induced non-photochemical fluorescence quenching [Y(NO)] showed that AgNPs inhibit the photo-protective capacity of PSII. AgNPs induce reactive oxygen species (ROS) that are mainly produced in the chloroplast. The activity of ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) was also very sensitive to AgNPs. The internalized Ag, regardless of whether the exposure was Ag(+) or AgNPs had the same capacity to generate ROS. Our results support the hypothesis that intra-cellular AgNP dissociate into high toxic Ag+. Rubisco inhibition leads to slowing down of CO₂ assimilation. Consequently, the solar energy consumption decreases and then the excess excitation energy promotes ROS generation in chloroplast.

Systematics and Evolution

Mitochondrial genome evolution in *Alismatales*: Size reduction and extensive loss of ribosomal protein genes

Petersen, G., Cuenca, A., Zervas, A., Ross, G.T., Graham, S.W., Barrett, C.F., Davis, J.I., Seberg, O. PLOS ONE 12: Article Number: e0177606 (2017)

The order Alismatales is a hotspot for evolution of plant mitochondrial genomes characterized by remarkable differences in genome size, substitution rates, RNA editing, retrotranscription, gene loss and intron loss. Here we have sequenced the complete mitogenomes of *Zostera marina* and *Stratiotes aloides*, which together with previously sequenced mitogenomes from *Butomus* and *Spirodela*, provide new evolutionary evidence of genome size reduction, gene loss and transfer to the nucleus. The *Zostera* mitogenome includes a large portion of DNA transferred from the plastome, yet it is the smallest known mitogenome from a non-parasitic plant. Using a broad sample of the Alismatales, the evolutionary history of ribosomal protein gene loss is analyzed. In *Zostera* almost all ribosomal protein genes are lost from the mitogenome, but only some can be found in the nucleus.

In silico identification of alternative oxidase 2 (AOX2) in monocots: A new evolutionary scenario

Costa, J.H., dos Santos, C.P., Lima, B.D.E., Netto, A.N.M., Saraiva, K.D.D.C., Arnholdt-Schmitt, B. JOURNAL OF PLANT PHYSIOLOGY 210: 58-63 (2017)

We identified AOX2 genes in monocot species from Lemnoideae (*Spirodela polyrhiza*, *Lemna gibba* and *Landoltia punctata*), Pothoideae (*Anthurium andraeanum* and *Anthurium amnicola*) and Monsteroideae (*Epipremnum aureum*) subfamilies within the Araceae, an early-diverging monocot family. These findings highlight the presence of AOX2 in the most ancient monocot ancestor and also that at least partial loss of this gene occurred during speciation events within several monocot orders. The presence of AOX2 in monocot species challenges (1) new understanding of the evolutionary history of the AOX gene family in angiosperms and (2) drives experimental and



bioinformatics efforts to explore functional relevance of the two AOX gene family members for plant growth and development. Knowledge gain in this field will impact running strategies on AOX-derived functional marker candidate development for plant breeding.



Links for further reading

<http://www.rduckweed.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 Fakhorian

<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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