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DUCKWEED FORUM

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International Steering Committee on Duckweed Research and Applications

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Lemna minor with varying genome size from adjacent ponds

Cover page

Lemna minor with varying genome size from adjacent ponds

Two ponds densely covered with *Lemna minor* near the historical cloister garden of the Naumburg cathedral in Saxony-Anhalt, Germany. The two ponds (in the photo and the inset) are only two meters apart but consist of *L. minor* plants with slightly different phenotypes and clearly different genome sizes. Photo credit: Dr. K. Sowjanya Sree, India; Inset: Dr. Klaus-J Appenroth, Germany.

In this issue

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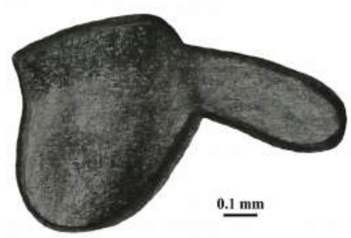
Science meets art: *Wolffia elongata* Landolt



Wolffia elongata Landolt was discovered by Elias Landolt and described by him in 1980. It is endemic to the very northern part of Southern America, Colombia and the island Curacao. Fronds are cylindrical in shape; the basal part floats just below the surface of the water, whereas the distal part is bent downwards and becomes submerged. It is well characterized by several molecular taxonomic investigations and is clearly separated from its next relative, *Wolffia columbiana*. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor:

Year of COVID-19.

Dear fellow duckweed researchers and practitioners,

I hope this letter finds you all safe and in good humor. Time seems to have flown by and 3 months have already passed since January when we last published the DF. However, so much seems to have transpired in this relatively short time that affected every corner of the inhibited portions of our planet. While I mentioned the emergence of the novel Coronavirus that causes COVID-19 in my last Editorial Letter, we have no idea of the dramatic impact and human cost that we have all witnessed over the past months and are still developing as I am writing this letter. The sheer scale of the economic impact to the world economy and the massive crisis that are beginning to erupt in developing countries extend far beyond the immediate health impact and mortality being reported in the news daily. The massive surge of unemployment in the U.S. that mirrors levels seen only during the Great Depression era is both shocking and worrisome. More than ever, our global communities are so intertwined in every way that it has become an existential necessity for countries to work closely together with key decisions driven by data and science instead of ideology and politics. This is clearly vital if we are to bring a sustainable closure to the pandemic that is currently plaguing the world. I think what this pandemic has shown is that it will be even more urgent for meaningful actions to minimize the impacts of the impending Climate Change for which no equivalent of a "vaccine" as a solution can even be conceived with our current technologies. To "act locally" while thinking about these global issues, I hope our community will work together to foster more cooperation and outreach efforts. Information and idea sharing are fundamental to scientific discourse and advances. As I see it, this is a key purpose of our newsletter, the *Duckweed Forum*. I hope to see more and more of you to take advantage of this platform to help build our global community by contributing your ideas and participating in polls.

As examples, in this issue of DF29, we have contributions from a student in the ETH at Switzerland, Melanie Binggeli, and also from Miri Lapidot et al. from Hinoman Ltd., a pioneering duckweed commercial venture in Israel. Melanie discussed her work focusing on potential food safety issues with producing fresh duckweed for human consumption while Miri and her collaborators gave a very comprehensive description of the oxalic acid concerns for fresh greens including various duckweed species. Both articles are of high interest to the development of duckweed as a food product and are in line with the surge of new companies that have been created in the past few years to deliver various food products from duckweed platforms. Similarly, the young student Bohan Yu from China also described her passion about translating her molecular studies with duckweed biodiversity to creating more sustainable animal feed in the Student Spotlight section. I think these articles effectively illustrate the international dimension of duckweed research and applications, as well as provide a sense of common purpose within our young community. I thus hope the Year of COVID-19 will heighten our awareness of the global need for collaboration and communication.

As always, the ISCDRA and I hope you will enjoy this issue of the DF. I especially like to acknowledge all the ad hoc contributors for their time and effort to make our DF truly a community platform for sharing.

Best wishes to all,

Eric Lam

Chair, ISCDRA

Oxalic acid content in Duckweeds

A case study of Mankai™ (*Wolffia globosa*) under intensive cultivation

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Oxalic acid concentrations in food crops have long been a concern in the human diet. This is because of the negative health effects associated with high intake of oxalic acid leading to the development of kidney stones, low plasma levels of iron and calcium, as well as hyposideremia and hypocalcemia (Noonan and Savage, 1999). Although cooking reduces the oxalic acid content in plant-based dishes, the increased trend of green smoothies consumption raises the concern of potential exposure to the intake of oxalate-rich foods such as spinach, collards, chard, beet leaves, kiwi, rhubarb (Noonan and Savage, 1999; EFSA, 2016). Despite this risk there is no established threshold of "Allowed Daily Intake" (ADI) for oxalic acid.

Mankai™ is a proprietary nutrient-rich super-vegetable strain of *Wolffia globosa*, part of a larger family of aquatic plants commonly referred to as duckweeds, hydroponically grown by Hinoman Ltd in a closely-controlled greenhouse facility. Mankai™ is intended to be marketed for human consumption as a food or food ingredient. The nutritional composition of Mankai™ has been found to be high in protein (40-50%) and dietary fibers (25-30%) and low in fat (7-10%). This study presents the low oxalic acid levels and lack of oxalate crystals in Mankai™ in comparison with various crop plants in general and with duckweeds in particular.

Oxalate presence in plants

Oxalate is a common compound found in many plants that are used as foods, such as nuts, fruits, vegetables, grains, and legumes, and is typically present as a salt or ester of oxalic acid $(COOH)_2$. Some plants such as *Oxalis* and *Rumex* species have a pH 2 cell sap due to acid oxalate $(HC_2O_4)^-$, primarily as acid potassium oxalate. In plants with a cell sap of approximately pH 6 the oxalate exists as oxalate ion $(C_2O_4^{2-})$, usually as soluble sodium oxalate and insoluble calcium and magnesium oxalates (Noonan and Savage, 1999). Calcium oxalate, $Ca(COO)_2$, a product of environmentally derived calcium ion (Ca^{2+}) and the metabolically formed oxalate ion from plants, is insoluble at a neutral or alkaline pH, but freely dissolves in acid, including in human stomachs. Only a few higher plant families show little or no oxalate accumulation (Zindler-Frank, 1976).

Oxalate may play various roles in plants as given below:

- Calcium regulation: calcium is essential for biological growth and development, but high concentrations are toxic to cells, and oxalate formation help to reduce calcium toxicity.
- Achieving ionic balance when there is an excess of inorganic cations represented by K^+, Na^+, NH_4^+, Ca^{2+} and Mg^{2+} over anions represented by NO_3^-, Cl^-, $H_2PO_4^-$, SO_4^{2-}): A theory has been advanced that organic acids accumulate as a result of excess cation concentrations in leaves. This is a consequence of nitrate transport from roots to shoots and subsequent reduction of nitrate to organic nitrogen in the leaves (Libert and Franceschi,1987; Prasad and

Shivay, 2017). Cations that originally accompanied nitrate from the roots are then balanced by the production of organic acids within the leaf and some (e.g. Ca-oxalate) may be immobilized by precipitation in the vacuole in the form of insoluble salts.

- Plant protection: The shapes of calcium oxalate crystals vary considerably and are typically described as raphides (needles), druses (spherical aggregates), crystal sand, styloids, and prisms (Cody and Horner, 1984; Horner and Wagner, 1995). It has been considered that oxalate crystals are a system of defense against herbivory, specifically for acicular crystals such as raphides and styloids (Webb, 1999).
- Heavy metal detoxification: Many plants absorb and accumulate heavy metals to relatively high concentrations without damage. Several mechanisms may contribute to heavy metal tolerance by plants including the metal deposition in vacuoles and incorporation of heavy metals in Ca oxalate crystals. Mazen *et al.* (2003) studied the accumulation of Cd, Pb and Sr in *Eichhornia crassipes* (water hyacinth), as this plant absorbs large amounts of heavy metals from aqueous systems. X–ray microanalysis of leaves indicates the progressive increase in Ca oxalate crystals during plant exposure to heavy metals (Mazen and El Maghraby, 1997).

The distribution of oxalates within plants varies within families, but the highest content is in leaves, followed by the seeds and it is the lowest in the stems (Noonan and Savage, 1999). Young leaves contain significantly more oxalates than the old leaves, with a 24% reduction in total oxalate occurred as the leaves matured. Young leaves of purslane (*Portulaca oleracea*) contain a mean of 482 mg total oxalate/100 g FW while leaves from older plants had 29% less (Palaniswamy, *et al.*, 2004). Based on these results it was recommended that young purslane leaves should not be harvested because of their higher oxalic acid content (Oscarsson and Savage, 2007). Oxalate concentration was closely associated with the length of the spinach growing period with a lower content following a short growing period and higher content in a longer growing period (Kaminishi and Nobuhiro, 2006). Therefore, it is possible that plant growth rate and thus the plant age could influence the level of oxalate accumulated in plant tissues.

Oxalate biosynthesis in plants

Efforts have been made to elucidate the metabolic pathways of oxalate biosynthesis and to reduce the oxalate levels in some crop plants (Libert and Franceschi, 1987). All the studies suggest that oxalates are one of the final products of metabolism.

Several pathways (Figure 1) were hypothesized for production of soluble and insoluble oxalates:

1) Photorespiratory glycolate/glyoxylate oxidation
2) Cleavage of ascorbate
3) Hydrolysis of oxaloacetate

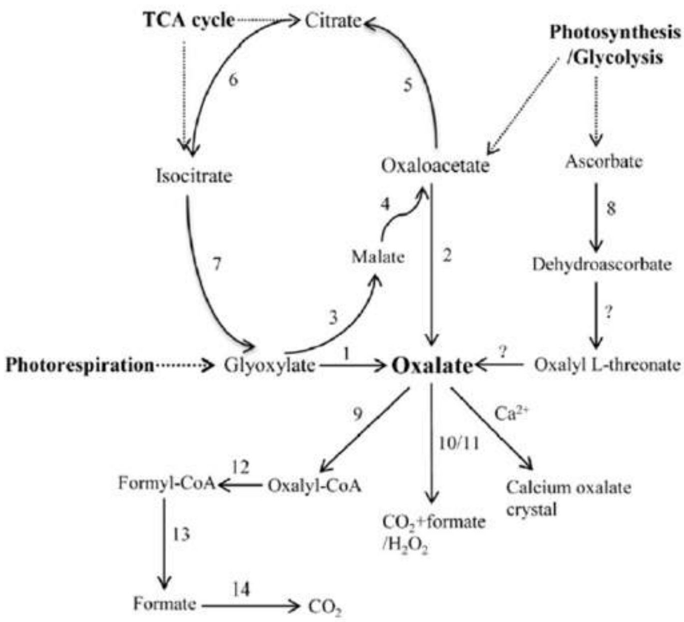

Figure 1. Schematic representation of putative biosynthetic and the degradation pathway of the oxalate (Cai et al., 2018). The enzyme names are as follows: (1) Glycolate oxidase (GLO); (2) Oxaloacetate acetylhydrolase (OXAC); (3) Malate synthase (MLS); (4) Malate dehydrogenase (MDH); (5) Citrate synthase (CTS); (6) Aconitase (ACO); (7) Isocitrate lyase (ICL); (8) Ascorbate peroxidase/Ascorbate oxidase (APX/AO); (9) Oxalyl-CoA synthetase (AAE3); (10) Oxalate decarboxylase (OXDC); (11) Oxalate oxidase (OXO); (12) Oxalyl-CoA decarboxylase (OXDE); (13) Formyl-CoA hydrolase (FXH); and (14) Formate dehydrogenase (FXDE).

Oxalate in Duckweeds

Like most commonly eaten vegetables (spinach, chives, beet leaves and parsley), duckweed can contain oxalic acid, which is the only identified compound produced by duckweed species that is toxic to animals at high levels (Stomp, 2005). In Duckweed family, the only studies for oxalic acid metabolism and calcium oxalate formation, were reported for *Lemna minor* and *Spirodela polyrhiza* (Mazen *et al.,* 2003; Kazumi, 1996; Franceschi,1989; Landolt, 1986; Saito 1995).

In *Lemna*, the majority of oxalic acid was found to be in the form of raphides, distributed in almost whole plant's body (Landolt, 1986) as well as in the roots (Franceschi,1987). According to this Landolt monograph, the raphides were not found in subepidermal layer and in the cells which are separating the air spaces vertically (Landolt, 1986). The amount of oxalic acid in *Lemna minor* was reported to be up to 4% of the dry weight, where 80% is found in the form of raphides, and located mainly in special cells called idioblasts (Bornkamm, 1965). In the case of *Spirodela polyrhiza,* it has been reported that there are two different kinds of crystals: druses (granular or star-like in shape) and raphides, in addition to the free oxalate (Landolt, 1986). Nevertheless, studies done on *Landoltia punctata*, formerly known as *S. punctata* (Ledbetter and Porter, 1970), and on *L. minor* (Arnott and Pautard, 1970) suggested that consumption of such plant species may pose low risk due to the raphides' presence as they do not cause irritation to humans. This is in contrary to the reports on the case of raphides from the Araceae plant family (Landolt, 1986).

In our study, we performed a scanning electron microscopy (SEM) combined with analytical electron microscopy analysis (Raman, 2014) to evaluate the presence of oxalate crystals in powdered duckweed species. The results show the existence of raphide crystals in the mixed powder sample of duckweed species (mainly *Lemna* sp. and *Spirodela* sp.), which is in line with previous studies (Figure 2). The raphide crystals were clearly observed throughout the sample outside the dry plant tissue.

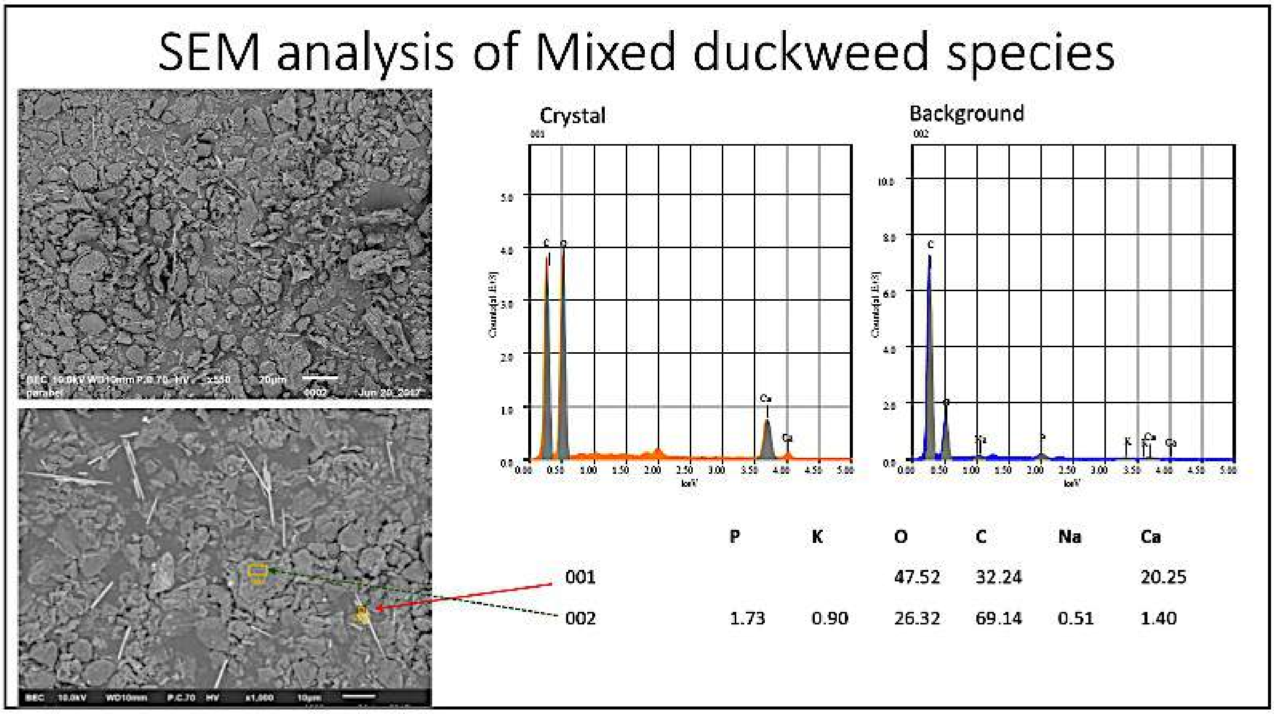

Figure 2: Scanning electron micrograph of mix powder of duckweed species. Image and analysis.

The arrows are pointing on the image site that was analyzed: 001 – Raphides crystals image analysis which validate the elemental composition of the oxalate crystals present; 002 – Organic material analysis as a background.

It is important to emphasize that while calcium oxalate crystals are formed in *Lemna* and *Spirodela* spp., *Wolffia* spp. was reported to contain a soluble form only, making *Wolffia* more suited for human consumption (Landolt & Kandeler, 1987). Following this, we analyzed oxalate in fresh biomass as well as in dry powder composed solely by one species of *Wolffia globosa* var. *Mankai*.

Oxalate in Mankai™

Mankai™ is a cultivated, proprietary strain of *Wolffia globosa* owned by Hinoman Ltd, Israel. We studied the potential formation of raphides in Mankai™. A dried powder form was subjected to SEM and analytical analysis and the results revealed no crystals detected in various tested samples (Figure 3).

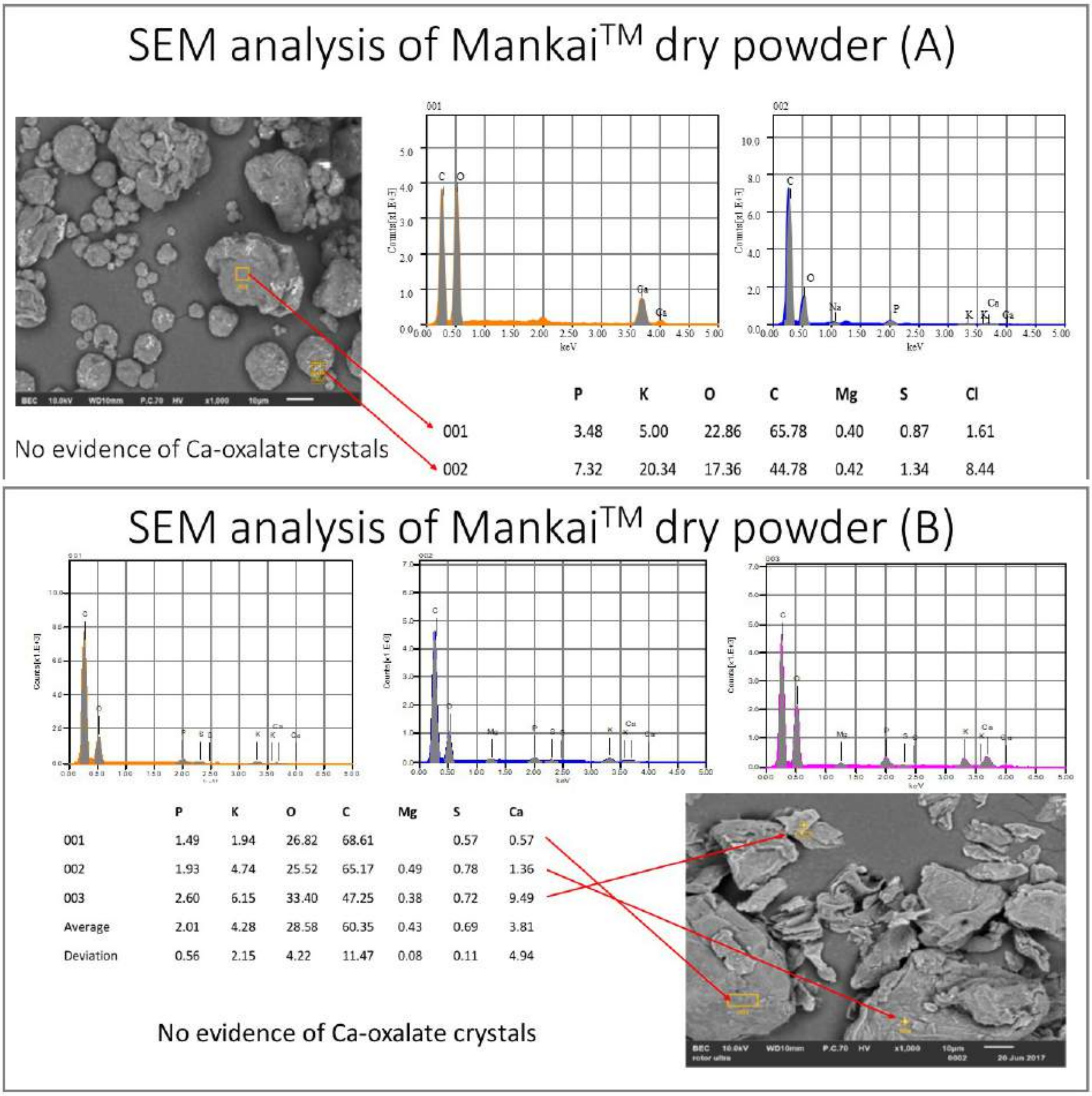

Figure 3: Scanning electron micrograph of Mankai™ dried powder in two different samples, (A) and (B). Image and analysis.

No raphides were detected in the images. The arrows point to image sites 001, 002 and 003 that were subjected to elemental analysis revealing a lack of crystals.

Oxalate biosynthesis in duckweed is amenable to some control by manipulating physical factors such as temperature, light and CO_2 (Landolt & Kandeler, 1987) and chemical growth conditions such as low magnesium and calcium in the culture media (Landolt & Kandeler, 1987; Franceschi, 1989). Calcium oxalate formation is a rapidly reversible process in duckweed (Franceschi, 1989).

Oxalic acid levels were measured in Mankai™ biomass grown in the closed controlled conditions of Hinoman's greenhouse facility using a HPLC (LC-DAD) method (AOAC 986.13 modified). Average amounts of 50mg/100g of oxalic acid in the fresh plants and 364 mg/100g of oxalic acid were found in the dried product. Comparison with other crops shows that the level found in Mankai™ is relatively low compared to most common vegetable used in a Western or Mediterranean diet (Table 1).

The oxalic acid content in dry powder of Mankai™ as a final food product revealed values in the range of 230-489 mg/100g. This range is much lower than the range of 5,300 -11,600mg/100g in a germplasm collection of dried spinach (Mou, 2008). We evaluated the oxalic acid content in Mankai™ products over a period of 3 years (2017-2019), and no fluctuation in different seasons was observed (Table 2).

Table 1: Comparison of oxalate content in fresh Mankai™ versus other green vegetables

Vegetable	mg/100g FW
Parsley	1700
Chives	1480
Amaranth	1090
Spinach	970
Lettuce	330
Broccoli	190
Celery	190
Asparagus	130
Cabbage	100
Pea	50
Mankai™	**50**
Cucumbers	20
Kale	20
Squash	20

Source: Values except for Mankai™ are from the USDA (1984)

Table 2: Oxalic Acid Content in Dried Mankai™ Powder

Lot processing date	Oxalic acid (mg/100g)
30 Jan. 2017	458
08 Mar. 2017	489
09 Apr. 2017	318
11 Jun. 2017	277
14 May 2018	230
25 Jul. 2018	279
14 Jan. 2019	424
08 Apr. 2019	436
Average	364

Analyses performed by Covance laboratory, Madison USA

In conclusion, while Ca-oxalate crystals have been found in various duckweed species, there is no evidence of Ca-oxalate in the *Wolffia globosa* var. Mankai™. The physical and chemical conditions of growth adopted by the Hinoman Ltd apparently limit oxalate concentration and bring it in line with the lowest levels of other green, edible vegetables. The amount of oxalic acid in Mankai™ is moderate, much lower than that found in green vegetables such as spinach, broccoli or lettuce.

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Opinion section: Understanding biological duckweed contaminations in human food

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Duckweed can serve as sustainable, healthy and delicious human nutrition. However, when growing duckweed for food applications, it needs to be considered that the conditions in the growing pond or other types of hydroponic systems are not only optimal for duckweed to grow, but also for a diverse variety of other organisms. A warm temperature, dissolved nutrients in the water and light can foster their growth as well. In some cases, this may not be an issue, because these organisms coexist without having adverse effects. But there are two categories of organisms that should be in the focus of everybody growing duckweed (or other fresh-water plants) as human food or animal feed.

The first category are those organisms which have a negative influence on duckweed itself, as an example by decreasing duckweed growth due to nutrient competition, or by nourishing itself from duckweed. The second category are organisms that are inappropriate for human consumption.


Photo made by Alexia Förster, 2020

If an organism of the first category makes its way into the pond and grows, this may – in the worst case – destroy the whole duckweed population in that pond. For example, we have heard of a company who closed down their business some decades ago, because a fungal disease made it into their growing system and destroyed their whole duckweed population within days. This is because starting from scratch with a small duckweed population means to wait a long time until reaching full growth capacity again. For a business, this can be very costly. Thus, it makes sense to keep a «backup culture» that is spatially separated from the growth system, so that a good population density can be reached faster when a new culture needs to be started again. Maintaining a good population density on its own inhibits growth of other organisms, as duckweed grows on the surface. This means the higher the density, the less light makes it through the plants into the growth medium. Keeping them too dense may create negative effects too, since individual plants may not receive sufficient light. Moreover, there is a so-called overcrowding effect which decreases growth rates because of fronds to fronds contact (Farber and Kandeler, J. Plant Physiol. 135: 94-98 (1989)). It thus makes sense to harvest often, in small portions, in order to keep the population density at an optimal level. This keeps both the population of other organisms down and increases the harvest. Further, a well-planned, thorough cleaning system needs to be in place, because a few cells of the undesirable organism left in the pond after cleaning can lead to a re-infection of the new duckweed crop, which leads to a new disease cycle.

In addition, adding the right amount of selected nutrients at the right time will also influence duckweed health, as the optimal balance is to have them well-nourished with all nutrients needed, but not over-nourished. Too low nutrition produces stress and could make them easier to attack by other organisms, while over-nourishing them would mean that they can't take up all the nutrients fast enough, which gives other competing organisms the opportunity to profit from these nutrients. (Yes, plants need "food" too, more than just light, water and CO_2! And as with humans, you need to have a nice balance. For plants, this is up to 17 different nutrients you should provide at the right time in the right amount.)

If an organism is of the second category and the infected duckweed is eaten, this can lead to human health issues. There are several organisms prone to cause adverse effects for human beings, e.g. human pathogens, the worst are those that we can't identify visually or through smell. It is thus of utmost importance to make sure that potentially adverse organisms are identified and controlled on a regular basis. In order to check whether the adverse organisms are not within the duckweed pond, one first needs to learn which harmful organisms could potentially occur in the ponds. First, research should be performed to understand what was already found in the past within duckweed ponds. Second, it needs to be understood what kind of adverse organisms live in the environment of the duckweed growing system, and which kind of entry points are possible. These organisms may vary, depending on the geographical location and the physical barriers of the growing system. Once these organisms are identified the question has to be raised to ascertain which way these organisms can sneak into the particular growth system. This can be influenced by the way the system interacts with the environment. As an example, when the duckweed is grown in a closed system, it's unlikely that ducks fly into the pond and transmit diseases adhering to their body. Thus, what we have left now is the sum of all potentially harmful organisms occurring in that specific geographical area, with preference on those that grow well in the conditions where the duckweed is grown, minus those that cannot enter the system due to physical or chemical barriers. For each of these organisms, a specific plan has to be determined to control on regular basis that this organism is not present at a significant level. Further, some organisms do occur in almost all systems but are not an issue as long as their concentration does not exceed a certain level. At this point in time, the other non-harmful organisms growing within the duckweed system matter: The more different organisms in the system covering a different niche, the more resilient the system against intruding organisms, the lower the risk of a sudden population explosion of a single intruder, and the higher the chance for a balanced leveling-out micro-ecosystem of the present organisms. (To find optimal organisms growing in synergy with duckweed and increasing the growth system resilience is another subject one could easily spend years doing research on.) For all organisms of the second category, regulatory food law of the geographical region has to be analyzed to understand the limit of CFU/g (colony forming units per gram of sample) allowed. Further, new measures may have to be taken, as the fresh-water environment differs from conventional soil-based systems and may thus require additional organisms to be tested which are not yet regulated by food laws.

One easy, economical way to check whether harmful organisms are present is to buy agar plates or nutrient broths containing a defined nutrient combination and providing an environment which benefits the organism at question (this has to be done individually for each organism at question). A duckweed sample can be homogenized, may be diluted based on a dilution series, and added to the agar plates or into the nutrient broth for incubation at a certain temperature for a defined time. After that, the CFU's can be counted (tedious!). Another approach is to pay considerably more and to have an accredited, ISO-certified laboratory doing this for you. For the test phase, as an example if you want to learn how fast a population of organism X increases over time when you start a new duckweed growing cycle from scratch, you can get your own agar plates and nutrient broths. If you want to sell duckweed as human food, you should do the analyses regularly with a professional lab. Having said this, it's also possible that there are organisms in your system which are difficult to get rid of, but which can be eliminated with the appropriate post-harvest treatment of the duckweed. As an example, if you cook the duckweed for a defined time at a defined temperature, or if you pasteurize it with high pressure, you can eliminate all organisms that are either temperature or pressure sensitive. This is usually the case for bacteria. However, some organisms can form super-dormant spores and certain fungi produce mycotoxins which are not eliminated with heat and remain in the food. This leads to a new research area: Each food product has its own biochemical and physical properties and is thus prone to fall for a different set of adverse organisms. For example, if you consume fresh duckweed, you should have a broad range of bacteria in mind that are harmful, exist in the environment and could potentially continue to grow on all kinds of fresh, uncooked vegetables, such as *Listerium monozytogenes*, *Escherichia coli*, *Pseudomonas* spp. et cetera. If you dry duckweed to a powder at a defined temperature and time, these bacteria are gone, but you should instead check, among others, for selected fungi that can recontaminate the product after drying, before packaging, and start to grow as soon as the product comes in contact with water again. This then leads towards optimizing the post-harvest process, such as developing a solution to sterilize the product right within the final packaging, which also influences final shelf-life. Unless there is a continuous overdemand, an increased shelf-life can be of benefit, as the final duckweed product does not have to be thrown away when it is not immediately sold.

Summa summarum, good hygienic conditions and a well thought out growth system can decrease contaminations that could have adverse effects on duckweed growth or human health. And a reasonable control as well as an optimized post-harvest process, depending on the properties of the duckweed product which will be sold, is necessary to assure food safety.

6th ICDRA 2021: 1st Circular

ISCDRA
International Steering Committee on Duckweed Research and Applications

Leopoldina
Nationale Akademie der Wissenschaften

gpz
Gesellschaft für Pflanzenzüchtung e.V.

IPK
LEIBNIZ-INSTITUT

6th ICDRA 2021
1st Circular

The 6th International Conference on Duckweed Research and Applications (6th ICDRA) will be held from **May 30 - June 02, 2021** in Gatersleben, Germany, at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK).

The **Conference website** is now available:

icdra-2021.ipk-gatersleben.de

and is open **for registration.**

(Student: 100.- €, Academic: 350.- €, Industry: 700.- €)

Abstract submission
authors may indicate their preference for short talk or poster presentation; the final decision will be made by the organizers in April 2021,

hotel booking between 48.- and 119.- € per night per person.

The **registration and abstract submission deadline is March 31, 2021.**
A reminder will be sent out in early March 2021.

If you need an official invitation or any other support, please contact the organisation committee as soon as possible to provide sufficient time for visa processing or other purposes.

We hope to meet you at the 6th ICDRA in Gatersleben.
The ISCDRA and the local organizers

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6th ICDRA

icdra-2021.ipk-gatersleben.de

Registration of duckweed clones/strains-Future Approach

Eric Lam, Klaus J. Appenroth, Yubin Ma, Tsipi Shoham, K. Sowjanya Sree

4th ISCDRA

The duckweed stock collections are required for long term maintenance of duckweed clones isolated from nature and to make them accessible for various research and developmental purposes (Sree and Appenroth, 2020). In the previous "Duckweed Forum" (ISCDRA, 2020) the "International Steering Committee on Duckweed Research and Applications" (ISCDRA) suggested to introduce a new "individual" registration system for duckweed clones. As a first step, we present here the details of 16 duckweed stock collections. These details will be updated from time to time and the data about other existing or new stock collections will be added on a timely basis.

We will keep the original clone registration system from Elias Landolt untouched in order to avoid confusion. It consists of four digits without any letters. The registration of clones with special physiological, genetic or morphological properties may be possible within this system also in future. This could be e.g. the case for whole genome sequenced clones. As suggested by Prof. Marvin Edelman, Weizmann Institute of Science, Israel, it may also be available for patent related clones. Please, contact Eric Lam, RDSC collection, Rutgers University, NJ, USA. The currently expanding as well as new clone collections should register their newly collected and maintained clones following an individualized registration system having the following format: two or three letter initials e.g., of the Principal Investigator or manager of the collection, followed by three or four digits (represented by 'x' in the table below).

Until now, the RDSC checked the reliability of species identification before registration. We request all managers of stock collections to determine the species identity with utmost accuracy using morphological criteria and molecular markers.

Table: Details of Duckweed Stock Collections across the globe.

S. No.	Director of the stock collection (Email ID)	Affiliation	Clone ID in individual system	Available clones with Landolt ID system	Available clones with individual ID system
1	Klaus J. Appenroth, Klaus.Appenroth@uni-jena.de	Matthias Schleiden Institute - Plant Physiology, University of Jena, Jena, Germany	KJAxxx	550 live, 36 herbarium specimens	50 (with KSS and BOG)

2	Manuela Bog, manuela.bog@uni-greifswald.de	Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany	BOGxxx	200	100 (with KJA)
3	Nikolai Borisjuk, nborisjuk@yahoo.com	Jiangsu Key Lab Ecoagr Biotechnol Hongze Lake, Huaian, China	NBxxxx	11	24
4	Hongwei Hou, houhw@ihb.ac.cn	Chinese Acad Sci, Inst Hydrobiology, Wuhan, Hubei, China	HHWxxx	14	5
5	Marcel Jansen, M.Jansen@ucc.ie	University College Cork, Earth and Environmental Sciences, Cork, Ireland	MJxxx	25	will be created
6	Jitendra P. Khurana, khuranaj@genomeindia.org	Dept. of Plant Molecular Biology, University of Delhi, South Campus, New Delhi, India	JPKxxx	12	8
7	Walter Laemmler, wlaemmler@duckweed.ch	Landolt Stock Collection, Zurich, Switzerland	only Landolt numbers	400 live, 5000 herbarium specimens	
8	Robert Laird, robert.laird@uleth.ca	Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada	RLxxx	5	70 (with RDSC and CCPCC)
9	Eric Lam, ericl89@hotmail.com	Rutgers Duckweed Stock Cooperative (RDSC), Rutgers University, NY, USA	ELxxx	740	50
10	Yubin Ma, mayubin@ouc.edu.cn	Ocean University of China, Qingdao, China	MYBxxxx	103	40
11	Viktor Olah, olahviktor@unideb.hu	Institute of Biology and Ecology University of Debrecen, Debrecen, Hungary	UDxxxx	7	34 (with MJ)

12	Ingo Schubert, schubert@ipk-gatersleben.de	Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany	only Landolt numbers	50	
13	K. Sowjanya Sree, ksowsree@gmail.com	Dept. of Environmental Science, Central University of Kerala, Periye, Kerala, India	KSSxxx	45 live, 36 herbarium specimens	27
14	Shuqing Xu, shuqing.xu@uni-muenster.de	Institute for Evolution and Biodiversity, University of Münster, Münster, Germany	XUxxx	150-160	5
15	Jiaming Zhang, zhangjiaming@itbb.org.cn	Institute of Tropical Bioscience and Biotechnology, Hainan, Haiku, China	ZJxxxx	50	700
16	Hai Zhao, zhaohai@cib.ac.cn	Chengdu Institute of Biology, Chinese Acad Sciences, Chengdu, China	ZHxxxx	640	150

References:

ISCDRA. 2020. Registration of duckweed clones/strains. *Duckweed Forum* 8: 6-7

Sree, K. S. and Appenroth, K-J. 2020. Worldwide genetic resources of duckweed: Stock collections. In: Cao, X.H. et al. (eds.), *The Duckweed Genomes*. Springer, pp. 39-46. ISBN 978-3-030-11045-1.

Student Spotlight: Bohan Yu

Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Hainan, 571101, China

(Email: yubohan.rayna@foxmail.com)

I am keen about the field of life science. Since I was a little girl, I had interest in small, lovely green plants. It was ideal when I decided to study this major. During my postgraduate study in Nanjing Agricultural University, I met my supervisor Dr. Jiaming Zhang who is an adjunct professor at the Nanjing Agricultural Univesity and a senior researcher at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences(CATAS), and started to focus on duckweed research. After I have got a Master's degree, I expect to be a professional devoted in this domain. Now I am working in Dr. Zhang's lab at the Institute of Tropical Bioscience and Biotechnology, CATAS and seeking an opportunity to study for a Ph. D degree.

Duckweed has strong adaptability, fast reproduction rate and high economic value. It can not only be used as medicine, but also as water cleaner and feed ingredient. The protein and starch content of duckweed is comparable to soybean, while its yield is far higher than that of soybean. The proportion of amino acids in duckweed is also excellent. At present, duckweed has been considered as one of the most potentially strategic new non-grain biomass raw materials.

My work is to study the biodiversity of duckweeds and development of duckweeds for bioenergy, including genetic diversity, phylogenetic analysis, nutritional ingredients and comprehensive utilization such as biogas fermentation and organic duckweed feed. The results of biogas fermentation showed that duckweed is an ideal raw material for anaerobic digestion with the maximum anaerobic digestion potential up to 195 ml/g TS. What's interesting about this research is not only the experiments, but also the collection of duckweed samples from all over the country. We look for duckweeds in different places like rivers, lakes and paddy fields in different provinces.

Ms. Bohan Yu havesting duckweed grown in wastewater from rubber processing factory

I have carried out analysis of genetic diversity of duckweed germplasms from Yunnan Province in detail. A total of 46 accessions of duckweeds were collected from Yunnan Province. Through identification based on molecular tools such as chloroplast *rps16* intron sequences and *atpF-atpH* spacer sequences, these germplasms were identified as belonging to 4 genera and 5 species, namely *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna perpusilla*, *Lemna aequinoctialis* and *Wolffia globosa*. Among the 5 species, *Lemna perpusilla* is a newly recorded species in southern China. Then we analyzed the nucleotide diversity indices, average number of nucleotide differences, haplotype diversity index and so on.

Most duckweed strains have high protein content, as well as good lysine and sulfur-containing amino acid content. When using duckweed as feed protein resource, the weight gain of tilapia fed on duckweed-based diet was higher than that on common feed. When the duckweed content was 20% and the feeding amount was 3% of the fish body weight, the tilapia weight gain rate reached 295.92±1.76%, which is 57.5% more than that using common feed, and the specific growth rate reached 3.44±0.01% per day at the end of the experiment. The protein content of duckweed feed mixed with this ratio increased by 4.3%, while the fat content decreased by 0.9%. The crude fiber content of duckweed feed increased but still within the national standard. The lysine content remained unchanged though the total amount of sulfur-containing amino acids, methionine and cystine, increased by 0.1%.

In a word, duckweed is a promising plant resource, which deserves further research and development. I am very satisfied studying on this unique plant that is awaiting exploration. I really wish that people can realize the significance of duckweed as a new strategic crop.

Ms. Bohan Yu Preparing duckweed planting stocks for massive field culture

From the Database

Highlights

Energy-efficient and environmentally friendly production of starch-rich duckweed biomass using nitrogen-limited cultivation

Guo, L; Jin, YL; Xiao, Y; Tan, L; Tian, XP; Ding, YQ; He, KZ; Du, AP; Li, JM; Yi, ZL; Wang, SH; Fang, Y; Zhao, H. (2020) JOURNAL OF CLEANER PRODUCTION 251: Article Number: 119726

Here, we combined the aquatic cultivation of the energy crop duckweed and nitrogen limitation to quickly produce starch-rich biomass. Under N limitation, duckweed can quickly accumulate starch at up to 52.37 ± 0.57% (dry weight). In the process, nitrogen use efficiency (NUE) reached 61.3 ± 1.8 kg biomass kg^{-1} N, exceeding that of most cereals. In this paper, we elucidated for the first time the mechanism of the high NUE of duckweed under N limitation. First, duckweed possesses higher N transport ability than most cereals and trees and can efficiently accumulate abundant N in vivo under N-sufficient condition. In addition, under N limitation, the stable expression and activity of key enzymes related to both carbon fixation and N assimilation ensured normal biomass accumulation. Furthermore, the efficient ubiquitin-proteasome system (UPS) and autophagy activated the endogenous N remobilization and recycling. Combined with the sharp increase in the expression and activity of duckweed glutamine synthase (GS), duckweed can efficiently redistribute and reuse the endogenous N to support its growth and starch accumulation. Especially, sewage was used to duckweed pre-cultivation in pilot-scale experiment, the approach can simultaneously achieve starch production and wastewater treatment. In a word, this paper provides an environmentally friendly, cost-effective and promising technology for starch production.

Duckweed hosts a taxonomically similar bacterial assemblage as the terrestrial leaf microbiome

Acosta, K; Xu, J; Gilbert, S; Denison, E; Brinkman, T; Lebeis, S; Lam, E (2020) PloS one 15: e0228560

Culture-independent characterization of microbial communities associated with popular plant model systems have increased our understanding of the plant microbiome. However, the integration of other model systems, such as duckweed, could facilitate our understanding of plant microbiota assembly and evolution. Duckweeds are floating aquatic plants with many characteristics, including small size and reduced plant architecture, that suggest their use as a facile model system for plant microbiome studies. Here, we investigated the structure and assembly of the duckweed bacterial microbiome. First, a culture-independent survey of the duckweed bacterial microbiome from different locations in New Jersey revealed similar phylogenetic profiles. These studies showed that Proteobacteria is a dominant phylum in the duckweed bacterial microbiome. To observe the assembly dynamics of the duckweed bacterial community, we inoculated quasi-gnotobiotic duckweed with wastewater effluent from a municipal wastewater treatment plant. Our results revealed that duckweed strongly shapes its bacterial microbiome and forms distinct associations with bacterial community members from the initial inoculum. Additionally, these inoculation studies showed the bacterial communities of different duckweed species were similar in taxa composition and abundance. Analysis across the different duckweed bacterial communities collected in this study identified a set of "core" bacterial taxa consistently present on duckweed irrespective of the locale and context. Furthermore, comparison of the duckweed bacterial community to that of rice and *Arabidopsis* revealed a conserved taxonomic structure between the duckweed microbiome and the terrestrial leaf microbiome. Our results suggest that duckweeds utilize similar bacterial community assembly principles as those found in terrestrial plants and indicate a highly conserved structuring effect of leaf tissue on the plant microbiome.

Biotechnology

Simultaneous biohydrogen production from dark fermentation of duckweed and waste utilization for microalgal lipid production

Mu, DM; Liu, H; Lin, WT; Shukla, P; Luo, JF (2020) BIORESOURCE TECHNOLOGY 302: Article Number: 122879

A cost-effective and environmentally friendly method for biofuel production was developed, by utilizing duckweed as feedstock for biohydrogen production through dark fermentation and simultaneously using the fermentative waste to produce microalgal lipids. The results suggested that acid hydrolysis (1% H_2SO_4) was more suitable for the pretreatment of duckweed biomass. Maximum hydrogen production of 169.30 mL g^{-1} dry weight was determined under a temperature of 35 °C and an initial pH of 7.0. After the dark fermentation, the volatile fatty acids (VFAs) including acetate and butyrate, were detected in the waste, with concentration determined as 1.04 g L^{-1} and 1.52 g L^{-1}, respectively. During the mixotrophic cultivation of *Chlorella sacchrarophila* FACHB-4 using waste as feedstock, the maximum microalgal biomass and the lipid productions were about 2.8 and 33 times higher with respect to the autotrophic growth. The simultaneous biohydrogen production and waste utilization method provided a green strategy for biofuel production.

Ecology

Habitat change and alteration of plant and invertebrate communities in waterbodies dominated by the invasive alien macrophyte *Lemna minuta* Kunth

Ceschin, S; Ferrante, G; Mariani, F; Traversetti, L; Ellwood, NTW (2020) BIOLOGICAL INVASIONS DOI: 10.1007/s10530-019-02185-5

The free-floating American duckweed, *Lemna minuta*, is an invasive species now widespread in Europe. Yet, its impact on freshwater ecosystems has been poorly investigated. In this study, the effects of the presence of this invasive duckweed on water quality, and aquatic plant and invertebrate communities were evaluated in sites in Central Italy. Water chemical and physical factors and community descriptors were analyzed to identify these effects. Surveys were carried out across 17 paired aquatic sites. Site pairs were similar in microclimate, hydrogeology and water quality, but differed in relation to the presence/absence of *L. minuta* floating mats. In sites with mats, light and dissolved oxygen in water were negatively correlated with increasing mat coverage and thickness. The limited light and hypoxic conditions under mats inhibited plant growth and had a selective impact on the invertebrate community. Sites with *L. minuta* had aquatic communities with a lower plant taxa richness and a contrasting composition, compared with those in sites without. At sites with mats some plants were unaffected, but the majority of plant taxa documented at sites without *Lemna* were no present at sites with *Lemna* or were very rare (macroalgae, submerged rhizophytes). As for invertebrates, hypoxic-tolerant taxa dominated under mats (*Ostracoda, Copepoda, Isopoda*), whilst those more sensitive to oxygen depletion, or obligate herbivores, or those with a winged stage or swimming on water surface, were rare or absent (*Ephemeroptera, Amphipoda, Chironomus, Notonecta*). *Lemna minuta* mats presence was associated with alterations in the underlying aquatic ecosystem, severely threatening the conservation of these habitats. Active management strategies, including spread-prevention techniques, or mechanical removal combined with biological control, are required to conserve these habitats.

Feed & Food

Production of selenium- and zinc-enriched *Lemna* and *Azolla* as potential micronutrient-enriched bioproducts

Li, J; Lens, PNL; Otero-Gonzalez, L; Du Laing, G (2020) WATER RESEARCH 172: Article Number: 115522

Selenium (Se) and zinc (Zn) are essential micronutrients that are often lacking in the diet of humans and animals, leading to deficiency diseases. *Lemna* and *Azolla* are two aquatic plants with a substantial protein content, which offer the possibility of utilizing them to remove Se and Zn from (waste)water while producing micronutrient-enriched dietary proteins and fertilizers. In this study, we explored interaction effects occurring between Se and Zn when these micronutrients are taken up by *Azolla* and *Lemna*. The two aquatic plants were grown on hydroponic cultures containing 0-5.0 mg/L of Se (Se(IV) or Se(VI)) and Zn. The Se and Zn content of the plants, growth indicators, bioconcentration factor (BCF) and Se/Zn removal efficiency from the water phase were evaluated. The results demonstrated that Se(IV) is more toxic than Se(VI) for both plant species, as evidenced by the remarkable decrease of biomass content and root length when exposed to Se(IV). Both aquatic plants took up around 10 times more Se(IV) than Se(VI) from the medium. Moreover, the Se accumulation and removal efficiency increased by 66-99% for Se(IV) and by 34-59% for Se(VI) in Lemna when increasing Zn dosage from 0 to 5.0 mg/L in the medium, whereas it declined by 13-26% for Se(IV) and 21-35% for Se(VI) in *Azolla*, suggesting a synergetic effect in *Lemna*, but an antagonistic effect in *Azolla*. The maximum BCF of Se in *Lemna* and *Azolla* were 507 and 667, respectively. The protein content in freeze-dried *Lemna* and *Azolla* was approximately 17%. The high tolerance and accumulation of Se and Zn in *Lemna* and *Azolla*, combined with their rapid growth, high protein content and transformation of inorganic to organic Se species upon Se(IV) exposure make *Lemna* and *Azolla* potential candidates for the production of Se(IV)- and Zn-enriched biomass that can be used as crop fertilizers or protein-rich food/feed supplements or ingredients. Accordingly, by growing the *Azolla* and *Lemna* on wastewater, a high-value product can be produced from wastewater while recovering resources.

Interaction with other organisms

Mutualistic Outcomes Across Plant Populations, Microbes, and Environments in the Duckweed *Lemna minor*

O'Brien, AM; Laurich, J; Lash, E; Frederickson, ME (2020) MICROBIAL ECOLOGY DOI: 10.1007/s00248-019-01452-1

The picture emerging from the rapidly growing literature on host-associated microbiota is that host traits and fitness often depend on interactive effects of host genotype, microbiota, and abiotic environment. However, testing interactive effects typically requires large, multi-factorial experiments and thus remains challenging in many systems. Furthermore, most studies of plant microbiomes focus on terrestrial hosts and microbes. Aquatic habitats may confer unique properties to microbiomes. We grew different populations of duckweed (*Lemna minor*), a floating aquatic plant, in three microbial treatments (adding no, "home", or "away" microbes) at two levels of zinc, a common water contaminant in urban areas, and measured both plant and microbial performance. Thus, we simultaneously manipulated plant source population, microbial community, and abiotic environment.

We found strong effects of plant source, microbial treatment, and zinc on duckweed and microbial growth, with significant variation among duckweed genotypes and microbial communities. However, we found little evidence of interactive effects: zinc did not alter effects of host genotype or microbial community, and host genotype did not alter effects of microbial communities. Despite strong positive correlations between duckweed and microbe growth, zinc consistently decreased plant growth, but increased microbial growth. Furthermore, as in recent studies of terrestrial plants, microbial interactions altered a duckweed phenotype (frond aggregation). Our results suggest that duckweed source population, associated microbiome, and contaminant environment should all be considered for duckweed applications, such as phytoremediation. Lastly, we propose that duckweed microbes offer a robust experimental system for study of host-microbiota interactions under a range of environmental stresses.

Synergistic *Lemna* Duckweed and Microbial Transformation of Imidacloprid and Thiacloprid Neonicotinoids

Muerdter, CP; LeFeyre, GH (2019) ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS 6: 761-767

Neonicotinoids are the most widely used insecticides in the world and are commonly measured in aquatic environments, including freshwater wetlands. We report for the first time the synergistic transformation of neonicotinoids by a *Lemna* duckweed and microbial system collected from an agricultural pond in Iowa, USA. Imidacloprid and thiacloprid were removed at statistically indistinguishable rates (0.63 ± 0.07 and 0.62 ± 0.05 d^{-1}), respectively) from hydroponic medium only when in the presence of both duckweed and its associated microbial community. As evidence for this duckweed-microbial synergy, experiments with surface-sterilized duckweed, duckweed-associated microbes, pond water microbes alone, and two other plant species (*Typha* sp. and *Ceratophyllum demersum*) did not yield significant neonicotinoid removal beyond initial biomass sorption. Degradation of imidacloprid and thiacloprid by the duckweed-microbial system generated multiple, known neonicotinoid metabolites (desnitro-imidacloprid, imidacloprid urea, thiacloprid amide, and 6-chloronicotinic acid). Measured metabolites with increased insect or vertebrate toxicity were either absent (imidacloprid olefin) or present only in small amounts (desnitro-imidacloprid; <1% of the parent). The neonicotinoid parent and metabolite mass balance did not fully account for total neonicotinoid removal, suggesting mineralization and/or other unidentified transformation products with unknown toxicity. This novel duckweed- and microbe-facilitated neonicotinoid degradation may represent an important contribution to the environmental fate of neonicotinoids.

Molecular Biology

Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed

Fu, L; Ding, Z; Tan, D; Han, B; Sun, X; Zhang, J (2020) BMC GENOMICS 21: 212

Salt significantly depresses the growth and development of the greater duckweed, *Spirodela polyrhiza*, a model species of floating aquatic plants. Physiological responses of this plant to salt stress have been characterized, however, the roles of long noncoding RNAs (lncRNAs) remain unknown. In this work, totally 2815 novel lncRNAs were discovered in *S. polyrhiza* by strand-specific RNA sequencing, of which 185 (6.6%) were expressed differentially under salinity condition. Co-expression analysis indicated that the trans-acting lncRNAs regulated their co-expressed genes functioning in amino acid metabolism, cell- and cell wall-related metabolism, hormone metabolism, photosynthesis, RNA transcription, secondary metabolism, and transport. In total, 42 lncRNA-mRNA pairs that might participate in cis-acting regulation were found, and these adjacent genes were involved in cell wall, cell cycle, carbon metabolism, ROS regulation, hormone metabolism, and transcription factor. In addition, the lncRNAs probably functioning as miRNA targets were also investigated. Specifically, TCONS_00033722, TCONS_00044328, and TCONS_00059333 were targeted by a few well-studied salt-responsive miRNAs, supporting the involvement of miRNA and lncRNA interactions in the regulation of salt stress responses. Finally, a representative network of lncRNA-miRNA-mRNA was proposed and discussed to participate in duckweed salt stress via auxin signalling. This study is the first report on salt-responsive lncRNAs in duckweed, and the findings will provide a solid foundation for in-depth functional characterization of duckweed lncRNAs in response to salt stress.

An efficient and rapid *Rhizobium rhizogenes* root transformation protocol for *Lemna minor*

Kanchanamala, RWMK; Bandaranayake, PCG (2019) PLANT BIOTECHNOLOGY REPORTS 13: 625-633

Duckweeds belong to the smallest aquatic flowering plant family, Lemnaceae, and have a rapid doubling time, making this group an excellent system to study reduced morphology and wide environmental adaptability at the molecular level. Despite the availability of genomic and transcriptomic data for duckweed member, *Lemna minor*, lack of an efficient genetic transformation system has limited its use in plant molecular biology research. The present study reports an efficient and rapid *Rhizobium rhizogenes*-mediated root transformation system for *L. minor*. Two different factorial experiments were designed to test the effect of explant type, age, culture media and inoculation methods on transformation efficiency. Leaf and root tip cut explants were inoculated with *R. rhizogenes* strain MSU 440 harboring pBIN-YFP vector using yellow fluorescent protein (YFP) as a reporter gene for identification of transgenic roots. In addition, two different culture media, full MS and 0.25X Hoagland, and four different infection methods, solid culture, centrifugation, liquid culture and sonication, were compared. After 8 weeks, about 17% of the root tip-cut explants infected via the solid culture method and maintained in 0.25X Hoagland medium had YFP-expressing roots. These transgenic *L. minor* roots were morphologically similar to normal roots and PCR analysis demonstrated that the YFP-expressing roots were positive for the integration-expected *rol* genes. The described optimized root transformation procedure is a valuable tool for pursuing high-throughput gene characterization studies in *L. minor*.

Agrobacterium-Mediated Transformation of *Lemna minor* L. with Hirudin and beta-Glucuronidase Genes

Kozlov, ON; Mitiouchkina, TY; Tarasenko, IV; Shaloiko, LA; Firsov, AP; Dolgov, SV (2019) APPLIED BIOCHEMISTRY AND MICROBIOLOGY 55: 805-815

The Agrobacterium-mediated transformation of a duckweed plant (*Lemna minor* L.) with the use of the organogenic callus and nucleotide sequence of a gene for hirudin-1 optimized for expression in plants has been performed. Eight transgenic plant lines transformed by hirudin and seven lines transformed by the gene for beta-glucuronidase were obtained. The expression of the glucuronidase gene was proven by histochemical staining and Western blot. ELISA of the transgenic plants showed that the content of beta-glucuronidase in them varied from 0.28 to 1.43% of the total soluble protein. The expression of the hirudin-1 gene was confirmed by RT-PCR, with the maximum hirudin accumulation being equal to 0.02% of the total soluble protein. The results of the study can be used in the development of an expression system using the duckweed plant to obtain hirudin and other recombinant proteins for pharmaceutical use.

The complete chloroplast genome of greater duckweed (*Spirodela polyrhiza* 7498) using PacBio long reads: insights into the chloroplast evolution and transcription regulation

Zhang, Y; An, D; Li, C; Zhao, Z; Wang, W (2020) BMC GENOMICS 21:76

Duckweeds (Lemnaceae) are aquatic plants distributed all over the world. The chloroplast genome, as an efficient solar-powered reactor, is an invaluable resource to study biodiversity and to carry foreign genes. The chloroplast genome sequencing has become routine and less expensive with the delivery of high-throughput sequencing technologies, allowing us to deeply investigate genomics and transcriptomics of duckweed organelles. Here, the complete chloroplast genome of *Spirodela polyrhiza* 7498 (SpV2) is assembled by PacBio sequencing. The length of 168,956bp circular genome is composed of a pair of inverted repeats of 31,844bp, a large single copy of 91,210bp and a small single copy of 14,058bp. Compared to the previous version (SpV1) assembled from short reads, the integrity and quality of SpV2 are improved, especially with the retrieval of two repeated fragments in ycf2 gene. There are a number of 107 unique genes, including 78 protein-coding genes, 25 tRNA genes and 4 rRNA genes. With the evidence of full-length cDNAs generated from PacBio isoform sequencing, seven genes (*ycf3, clpP, atpF, rpoC1, rpl2, rps12* and *ndhA*) are detected to contain type-II introns. The *ndhA* intron has 50% more sequence divergence than the species-barcoding marker of *atp*F-*atp*H, showing the potential power to discriminate close species. A number of 37 RNA editing sites are recognized to have cytosine (C) to uracil (U) substitutions, eight of which are newly defined including six from the intergenic regions and two from the coding sequences of rpoC2 and *ndh*A genes. In addition, nine operon classes are identified using transcriptomic data. It is found that the operons contain multiple subunit genes encoding the same functional complexes comprising of ATP synthase, photosynthesis system, ribosomal proteins, et.al., which could be simultaneously transcribed and coordinately translated in response to the cell stimuli. The understanding of the chloroplast genomics and the transcriptomics of *S.polyrhiza* would greatly facilitate the study of phylogenetic evolution and the application of genetically engineering duckweeds.

Physiology & Stress

Elucidating physiological and biochemical alterations in giant duckweed (*Spirodela polyrhiza* L. Schleiden) under diethyl phthalate stress: insights into antioxidant defence system

Sharma, R; Kaur, R (2020) PEERJ 8: Article Number: e8267

The emollient properties of phthalates have led to their extensive production and intense use in plastic products. Owing to their weak covalent bonding with the plastic polymers, phthalates enter into the environment during their manufacturing, processing, disposal, consequently found their way directly into water sources, soil, and sediments. The present study envisaged the toxic *effects of diethyl phthalate (DEP) on physiological and biochemical attributes of Spirodela* polyrhiza, when exposed to various concentrations of DEP (0, 10, 20, 40, 80,100, 200, and 400 ppm) for short term exposure period of seven days. Plants of *S. polyrhiza* accumulated significant amount of DEP (112 mg kg^{-1} fw) when exposed to various concentrations of DEP for seven days. Results depicted that DEP toxicity significantly ($p \leq 0.05$) affected growth parameters and pigments in treated *S. polyrhiza* as compared to control. Further, high doses of DEP (400 ppm) caused significant decrement in carbohydrate (86%), protein (76%) and elevation in MDA content (42%). Meanwhile, DEP altered the activities of antioxidant enzymes (SOD, CAT, APX, GPX and GR) along with the induction of enhanced levels of proline, electrolyte leakage and phenolic content. Scanning electron microscopic and confocal studies also confirmed oxidative stress in plants under DEP stress. Present findings will help understand the accumulation, tolerance, and detoxification mechanisms of DEP by *S. polyrhiza*.

Accumulation, morpho-physiological and oxidative stress induction by single and binary treatments of fluoride and low molecular weight phthalates in *Spirodela polyrhiza* L. Schleiden

Sharma, R; Kumari, A; Rajput, S; Nishu; Arora, S; Rampal, R; Kaur, R (2020) SCIENTIFIC REPORTS 9: Article Number: 20006

The present study examined the interactive effects of fluoride and phthalates on their uptake, generation of reactive oxygen species and activation of antioxidative defence responses in *Spirodela polyrhiza* L. Schleiden. A hydroponic study was conducted in which *S. polyrhiza* cultured in Hoagland's nutrient medium, was exposed to fluoride (50 ppm) and different concentrations viz., 75, 150 300 ppm of diethyl phthalate (DEP) and diallyl phthalate (DAP) individually as well as in combination for the time period of 24, 72, 120 and 168 h respectively. A significant decline in fresh weight, dry to fresh weight ratio, total chlorophyll, carotenoid content and increased anthocyanin content was observed. Fluoride and phthalates was found to be readily accumulated by S. *polyrhiza* in all the exposure periods. Interestingly, when binary treatments were given in nutrient medium, uptake of both fluoride and phthalate was found to be influenced by each other. In combined treatments, DEP stimulated fluoride uptake, while its own uptake was restricted by fluoride. In contrary to this, fluoride stimulated DAP uptake. Moreover, combined stress further caused significant decrement in carbohydrate, protein content and increment in MDA levels, phenolic content and electrolyte leakage. Nevertheless, phthalates showed more pronounced oxidative stress and growth inhibition compared to fluoride. To cope up with the oxidative damage, enhanced level of antioxidant enzymatic activities was observed in *S. polyrhiza* under both fluoride and phthalate stress as compared to control. Scanning electron microscope imaging of leaf stomata revealed that combined stress of fluoride with phthalates caused distortion in the shape of guard cells. Confocal micrographs confirmed the generation of reactive oxygen species, cell damage, disruption in membrane integrity, and enhanced levels of glutathione in plant cells. This study focussed on ecotoxicological and interactive significance of fluoride led phthalate uptake or vice versa which was also assumed to confer tolerance attributes.

Lemna minor recovery potential after short-term exposure to sulfonylurea herbicide

Zaltauskaite, J; Kaciene, G (2020) ACTA PHYSIOLOGIAE PLANTARUM 42: Article Number: 35

We have investigated the effects of the sulfonylurea herbicide Sekator OD 375 on common duckweed (*Lemna minor L.*) and its potential to recover after the herbicide exposure. The plants were exposed to Sekator OD 375 for a 7-day period at the concentrations ranging from 5 to 100 μL L^{-1}, equivalent to 0.005-0.1 of field application rate. The treated plants were transferred to clean fresh growth medium for a 7-day period to observe the plants recovery. The exposure to herbicide significantly reduced the growth rate and the biomass of common duckweeds (*L. minor*), lowered the content of photosynthetic pigments and induced membrane lipid peroxidation. After the transfer to fresh, clean growth media, the plants exposed to concentrations higher than 50 μL L^{-1} of herbicide showed no potential to recover. *L. minor* exposed to relatively low levels of herbicide (below 50 μL L^{-1}) showed a potential to recover their new fronds production: the relative growth rate in the recovery phase was higher than in the exposure period. However, after the recovery phase, the final biomass of the exposed plants was below initial values. Exposure to the herbicide-induced membrane lipid peroxidation (measured as the concentration of malondialdehyde). Thus, plants failed to recover their membranes during the recovery phase and a further increase in the concentrations of malondialdehyde was observed.

Phytoremediation

The importance of the calcium-to-magnesium ratio for phytoremediation of dairy industry wastewater using the aquatic plant *Lemna minor* L.

Walsh, E; Paolacci, S; Burnell, G; Jansen, MAK (2020) INTERNATIONAL JOURNAL OF PHYTOREMEDIATION DOI: 10.1080/15226514.2019.1707478

Lemnaceae are being exploited to remediate a variety of different wastewaters. Our aim was to remediate dairy processing waste, which is produced in large amounts, and contains valuable plant nutrients, for example, nitrate, ammonium, phosphate and iron. However, initial trials failed to establish the growth of *Lemna minor* on this medium. A lack of growth can be due to a lack of essential plant nutrients, or the presence of phytotoxic ingredients. In this study we show that not just nutrient concentrations, but also the ratios between them can be important in facilitating growth. Lab-scale experiments in which *L. minor* were grown on 100 mL of synthetic dairy industry wastewater demonstrated that the skewed Ca:Mg ratio in synthetic wastewater is a key obstacle to good growth. Experiments showed that a ratio which favours magnesium over calcium negatively affects *L. minor* growth and photosynthetic yield, leading to RGRs as low as 0.05 d^{-1}. A change in this ratio to favour calcium, through the addition of calcium sulphate, leads to RGRs of 0.2-0.3 d^{-1}. Experiments lead us to conclude that a Ca:Mg ratio of 1:1.6 or greater is necessary for *L. minor* growth, and therefore phytoremediation of dairy industry processing wastewater.

Phytoremediation capabilities of *Salvinia molesta*, water hyacinth, water lettuce, and duckweed to reduce phosphorus in rice mill wastewater

Kumar, S; Deswal, S (2020) INTERNATIONAL JOURNAL OF PHYTOREMEDIATION DOI: 10.1080/15226514.2020.1731729

The objective of this study was to investigate the reduction of phosphorus from rice mill wastewater by using free floating aquatic plants. Four free floating aquatic plants were used for this study, namely water hyacinth, water lettuce, salvinia, and duckweed. The aquatic plants reduced the total phosphorus (TP) content up to 80% and chemical oxygen demand (COD) up to 75% within 15 days. The maximum efficiency of TP and COD reduction was observed with water lettuce followed by water hyacinth, duckweed, and salvinia. The study also aims to predict phosphorus removal by three modeling techniques, for example, linear regression (LR), artificial neural network (ANN), and M5P. Prediction has been done considering hydraulic retention time (HRT), hydraulic loading rate (HLR), and initial concentration of phosphorus (C-in) as input variables whereas the reduction rate of TP (R) has been considered as a predicted variable. ANN shows promising results as compared to M5P tree and LR modeling. The model accuracy is analyzed using three statistical evaluation parameters which are coefficient of determination (R^{-2}), root mean square error (RMSE), and means absolute error (MAE).

Phytoremediation potential of the duckweeds *Lemna minuta* and *Lemna minor* to remove nutrients from treated waters

Ceschin, S; Crescenzi, M; Iannelli, MA (2020) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH DOI: 10.1007/s11356-020-08045-3

Phytoremediation potential of duckweeds (*Lemna minuta, Lemna minor*) to remove nutrients from simulated wastewater was analyzed. In two separate experiments, the two species were grown for 28 days in waters enriched with nitrate and phosphate to simulate nutrient concentrations of domestic wastewater. Water physical and chemical measurements (temperature, pH, conductivity, oxygen) and plant physiological and biochemical analysis (biomass, relative growth rate-RGR, nutrient and chlorophyll contents, peroxidative damage, bioconcentration factor-BCF) were made to test and compare the phytoremediation capacity of the two *Lemna* species. *L. minuta* biomass increased almost tenfold during the time-course of the treatment resulting in a doubling of the mat thickness and a RGR of 0.083 ± 0.001 g/g day. Maximum frond content of phosphate was reached by day 21 (increase over 165%) and nitrate by day 7 (10%). According to the BCF results (BCF > 1000), *L. minuta* was a hyperaccumulator for both nutrients. On the other hand, *L. minor* biomass and mat thickness decreased continuously during incubation (RGR = - 0.039 ± 0.004 g/g day). In *L. minor* fronds, phosphate content increased until day 14, after which there was a decrease until the end of the incubation. Frond nitrate content significantly decreased by day 7, but then remained relatively constant until the end of the experiment. *L. minor* proved to be hyperaccumulator for phosphates, but not for nitrates. Results indicated *L. minuta* has a greater potential than *L. minor* to remove both nutrients by bioaccumulation, especially phosphates, demonstrated also by better physiological and biochemical responses. However, during the incubation, the chlorophyll content of *L. minuta* mat did continuously decrease and peroxidative damage had increased until day 14, indicating that the system was under some kind of stress. Strategies to avoid this stress were discussed.

An assessment of the effect of *Lemna minor, Cyanobacteria Oscillatoria* Sp and aeration on the elimination of cadmium, nickel and zinc from urban and industrial wastewater

Abadi, SAH; Baharlouei, J; Najafi, P; Ghahsareh, AM (2020) INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY DOI: 10.1080/03067319.2020.1724983

Considering the serious shortage of drinking water in Iran and the need to use other sources of water including wastewater, blackwater and saltwater in agriculture, in the present study, the effect of common duckweed (*Lemna minor*) and filamentous *Cyanobacteria Oscillatoria* Sp on purification of urban wastewater (Khorasgan University wastewater) and industrial wastewater (Esfahan Sugar Co. wastewater) samples with and without aeration in glass aquariums for 15 days with 5-day gaps in between will be analysed; and the density factors of cadmium, nickel and zinc are measured first for the sample urban and industrial wastewaters and later in each step of the factorial experiment. Then, the data are assessed in SAS using randomised complete block design for factorial experiment in three repetitions and Duncan's new multiple range test. The results indicate that *Cyanobacteria Oscillatoria* Sp had the best effect on reducing nickel in industrial wastewater without aeration (between day 10 and 15). *Lemna minor* was better than Cyanobacteria Oscillatoria Sp in reducing zinc in industrial wastewater (the best performance was from the first 5 days) and after purification, this water will cause no problems in terms of zinc. The treatments did not have predictable behaviour in reducing cadmium but cadmium levels in both types of wastewaters were not so bad as to cause irrigation problems.

Feasible Green Strategy for the Quantitative Bioaccumulation of Heavy Metals by *Lemna minor*: Application of the Self-Thinning Law

Sun, Y; Gao, PK; Ding, N; Zou, XM; Chen, YL; Li, TH; Wang, CT; Xu, X; Chen, TT; Ruan, HH (2020) BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 104: 282-287

This study involved the development of mathematical linear regression models to describe the relationships between mean plant biomass (M) and population density (D), M and frond diameter (L), frond numbers (N) and L of *Lemna minor* under different initial population densities (3200, 4450, and 6400 plants m^{-2}), respectively, from the perspective of the self-thinning law. Our results revealed that the value of the allometric exponents for M and D were - 3/2. Further, the concentrations of Zn, Pb, Cu, Fe, and Ni accumulated in *L. minor* plants were 0.86, 0.32, 0.36, 0.62, and 0.39 mg kg^{-1} respectively. Based on these developed equations and the heavy metal accumulations by *L. minor*, the phytoremediation capacity of *L. minor* was quantified via its frond diameters. Overall, the present study provides a cost-effective green method for managing the phytoremediation of heavy metal-contaminated aquatic environments.

Removal of tetracycline antibiotic by *Lemna gibba* L. from aqueous solutions

Topal, M; Obek, E; Senel, GU; Topal, EIA (2020) WATER AND ENVIRONMENT JOURNAL 34: 37-44

In this study, the efficiency of Lemna gibba L. on tetracycline (TC) removal from the solutions prepared at various initial TC concentrations (50, 100 and 300 µg/L) was investigated. The study was conducted in the reactors planted with *Lemna gibba* L. plants. *Lemna gibba* L. plants in the reactors were harvested at various hydraulic retention times. Then, the concentrations of TC were determined by using LC/-MS-MS. The maximum removal efficiencies for the TC50, TC100 and TC300 in the planted reactors were 99.8 ±4.1; 99.9 ± 4.9 and 99 ± 4.9%, respectively. The harvesting times for *Lemna gibba* L. at TC50, TC100 and TC300 concentrations were determined to be 12, 8 and 12 hours, respectively. First-, second- and pseudo-first-order kinetics are determined in the planted reactors (with *Lemna gibba* L.). According to the study results, second-order kinetics were obtained at TC50, TC100, TC300 concentrations. As a result, the *Lemna gibba* L. plant can be used as an alternative treatment method to other advanced treatment methods and it can be done with a cheap method by adapting to existing treatment plants. *Lemna gibba* L. plants can be used to remove pollutants by applying them to polluted lakes and water bodies.

Phytotoxicity

Toxicological effects of AgNPs on duckweed (*Landoltia punctata*)

Lalau, CM; Simioni, C; Vicentini, DS; Ouriques, LC; Mohedano, RA; Puerari, RC; Matias, WG (2020) SCIENCE OF THE TOTAL ENVIRONMENT 710: Article Number: 136318

Silver nanoparticles (AgNPs) are widely applied in several types of products since they act as a biocide. However, their high level of release into the environment can bring risks to ecosystems. Thus, the toxicity of AgNPs toward duckweed (*Landoltia punctata*) was investigated by monitoring the growth rate inhibition and the effect on the photosynthetic metabolism through morphological and ultrastructural analysis. The AgNPs were characterized by transmission electron microscopy and the effective diameter (dynamic light scattering) and zeta potential were determined. Plants were grown according to the environmental conditions recommended in ISO/DIS 20079 and then exposed to different concentrations of AgNPs. Inhibition of the growth rate was measured based on the EC_{50} and changes in the morphology, cellular structures and photosynthetic pigments were evaluated along with the silver accumulation. Although the results showed low growth inhibition when compared to other studies, significant damage to the ultrastructure, decreases in the photosynthetic pigments and starch grains, an increase in the phenolic compounds and physiological changes, such as a loss of color, were observed. Moreover, the accumulation of silver ions was noted and this could lead to bioamplification in consumer organisms, since duckweed belongs to the first level of the food chain.

Investigations on the *uptake and transformation of sunscreen ingredients in duckweed (Lemna* gibba) and *Cyperus altermfolius* using high-performance liquid chromatography drift-tube ion-mobility quadrupole time-of-flight mass spectrometry

Seyer, A; Mlynek, F; Himmelsbach, M; Buchberger, W; Klampfl, CW (2020) JOURNAL OF CHROMATOGRAPHY A 1613: Article Number: 460673

The uptake, translocation and transformation of three UV-blockers commonly employed in sunscreens, namely avobenzone, octocrylene and octisalate from water by *Lemna gibba* and *Cyperus alternifolius* was investigated. Reversed phase high performance liquid chromatography coupled to drift-tube ion-mobility quadrupole time-of-flight mass spectrometry was used for analyzing the extracts from the selected plants after incubation with the UV-blockers for one week. For avobenzone several transformation products resulting from hydroxylation, demethylation and oxidation of the parent molecule could be identified by measuring accurate mass, performing MS/MS experiments and by determining their drift-tube collision cross sections employing nitrogen as drift gas. In addition, the plants were subjected to two commercially available sunscreens, providing similar results to those obtained for the standard solutions of the UV-blockers. Finally, a kinetic study on the uptake and transformation of avobenzone, octocrylene and octisalate was conducted over a period of 216 h, revealing that the UV-filters were mostly present in their parent form and only to a smaller part converted into transformation products.

Phytotoxicity

Biosorptive removal of acid orange 74 dye by HCl-pretreated *Lemna* sp.

Reyes-Ledezma, JL; Uribe-Ramirez, D; Cristiani-Urbina, E; Morales-Barrera, L (2020) PloS one 15:e0228595

Acid orange 74 (AO74) is a chromium-complex monoazo acid dye widely used in the textile industry. Due to being highly toxic and non-biodegradable, it must be removed from polluted water to protect the health of people and the environment. The aim of this study was two-fold: to evaluate the biosorption of AO74 from an aqueous solution by utilizing HCl-pretreated *Lemna* sp. (HPL), and to examine dye desorption from the plant material. The maximum capacity of AO74 biosorption (64.24 mg g^{-1}) was reached after 4 h at the most adequate pH, which was 2. The biosorption capacity decreased 25% (to 48.18 mg g^{-1}) during the second biosorption/desorption cycle and remained essentially unchanged during the third cycle. The pseudo-second-order kinetics model concurred well with the experimental results of assays involving various levels of pH in the eluent solution and distinct initial concentrations of AO74. NaOH (0.01 M) was the best eluent solution. The Toth isotherm model best described AO74 biosorption equilibrium data. FTIR analysis confirmed the crucial role of HPL proteins in AO74 biosorption. SEM-EDX and CLSM techniques verified the effective biosorption/desorption of the dye during the three cycles. Therefore, HPL has potential for the removal of AO74 dye from wastewaters.

Taxonomy

Duckweed (Lemnaceae): Its Molecular Taxonomy

Bog, M; Appenroth, KJ; Sree, KS (2019) FRONTIERS IN SUSTAINABLE FOOD SYSTEMS 3: Article Number: UNSP 117

Duckweeds include the world's smallest and fastest growing flowering plants that have the capacity to produce huge biomass with a broad range of potential applications like production of feed and food, biofuel and biogas. In order to achieve optimal and sustainable commercial system, it is necessary that suitable species and clones of duckweeds be identified and selected based on appropriate strategies. However, a high degree of reduction in their structural complexity poses serious problems in identification of closely related species of duckweeds, on a morphological basis. Use of molecular taxonomic tools is the present solution. The state of the art of molecular taxonomy of all the five genera of duckweeds (*Spirodela, Landoltia, Lemna, Wolffiella*, and *Wolffia*) is based mainly on the techniques of fingerprinting by amplified fragment length polymorphism (AFLP) and barcoding using sequences of plastidic DNA fragments. After more than 15 years of molecular taxonomic investigations, a certain viewpoint is now available demonstrating all five genera to be monophyletic. Also, the phenetic analyses had made huge progress in delineating the currently defined 36 species of duckweeds, although, all species cannot yet be defined with confidence. *Wolffiella* has turned out to be the most complicated genus as only 6 to 7 species out of the 10 can be reliably delineated. Further progress in the phylogenetic and phenetic analyses requires more advanced methods like next generation and/or whole genome sequencing. First results using the method genotyping-by-sequencing in the genus *Lemna* (in combination with metabolomic profiling by matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MALDI-TOF-MS) as well as AFLP and barcoding by plastidic sequences) are more promising: The species *Lemna valdiviana* and *Lemna yungensis* were united to one species, *Lemna valdiviana*. This reduced the total number of Lemnaceae species to 36.

De novo assembly, transcriptome characterization, and simple sequence repeat marker development in duckweed *Lemna gibba*

Fu, LL; Ding, ZH; Kumpeangkeaw, A; Tan, DG; Han, BY; Sun, XP; Zhang, JM (2020) PHYSIOLOGY AND MOLECULAR BIOLOGY OF PLANTS 26: 133-142

Lemna gibba is a species of duckweed showing great potential in bioenergy production and wastewater treatment. However, the relevant transcriptomic and genomic resources are very limited for this species, which dramatically hinders its genetic diversity and genome mapping researches. In this work, ~ 233.5 million clean reads were generated from *L. gibba* by Illumina paired-end sequencing, and subsequently they were de novo assembled into 131,870 unigenes, of which 61,622 were annotated and 43,319 were expressed with Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) > 5. In total, 19,297 simple sequence repeats (SSRs) were identified from 15,261 SSR-containing unigenes. Dinucleotide (78.4%) were the most abundant SSRs, followed by tri- (14.9%), tetra- (4.1%), and penta-nucleotides (1.5%). The top three motifs were AG/CT (69.9%), AC/GT (6.5%), and ATC/ATG (4.9%). Further analysis revealed that the presence of SSR motif was independent of the expression level for a given gene. Based on the sequence of these SSR-containing unigenes, a total of 10,292 SSR markers were developed, of which only 2671 were further retained after removing those derived from unannotated or extra-low expressed (e.g., FPKM ≤ 5) unigenes. Finally, a subset of 70 SSR markers was randomly selected and examined in nine diverse *L. gibba* genotypes for the PCR amplification and polymorphism, as well as in other duckweed species for the inter-specifically amplifiability. This work is the first report on the transcriptome-based large-scale SSR markers development and analysis in *L. gibba*. The transcriptome generated and the SSR markers developed in this work will provide a valuable resource for genetic diversity assessment in *L. gibba* and also for species relationship investigation in Lemnaceae family.

Unusual Applications

Whitening and anti-wrinkle effect of *Spirodela polyrhiza* extracts

Kim DH; Park TS; Kim SG (2019) JOURNAL OF APPLIED BIOLOGICAL CHEMISTRY 62: 391-398

The antioxidant, whitening, and anti-wrinkle activity of *Spirodela polyrhiza* extracts and fractions were evaluated to determine its efficacy as a functional cosmetic material. 1,1- diphenyl-2-picrylhydrazyl and 2,2'-azinobis-3-ethylbenzothiazoline- 6-sulfonic acid radical scavenging activities were 44.2 and 74.3%, respectively, at 100 μg/mL of SE-E (the ethyl acetate fraction of 70% ethanol extract). To measure anti-wrinkle effects, procollagen biosynthesis and matrix metalloproteinase-1 (MMP-1) inhibition activity were determined. At 25 μg/mL of SE (70% ethanol extract), the biosynthesis activity was 48.5%, and SE-E showed the best activity (57.8%) at the same concentration. MMP-1 inhibition activity of SE and SE-E was 13.4 and 28.5%, respectively, at 25 g/mL. Finally, the inhibition of cellular melanin synthesis and cellular tyrosinase were measured to determine the whitening effect; at 25 μg/mL, the inhibition activities of SE were 9.6 and 13.8%, respectively, and those for SE-E were 15.4 and 22.0%, respectively. Our results confirmed the possibility of SE and SEE as effective functional materials. Further research investigating the antimicrobial, anti-inflammatory, and anticancer activities of *S. polyrhiza* is necessary to confirm its potential use in the food, cosmetics, and drug industries.

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

In addition to invitees by a Duckweed Steering Committee member, if you are considering submitting a contribution to DF but are unsure about the fit of your idea, please feel free to contact one of the members in the Duckweed Steering Committee in order to obtain feedback as to the appropriateness of the subject for DF. Please include a few sentences describing the overall topic that you are interested to present on, and why you think it is of interest to the general duckweed community. If you have the abstract or draft text prepared, please include it. The Duckweed Steering Committee will discuss the material in one of its meetings and the decision to formally invite submission will be given shortly afterwards.

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Formatting requirements:

- A commonly used word processing program, such as Word, is highly recommended.

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- Single-spaced text throughout.
- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
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- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
- Should avoid acronyms or abbreviations aside from the most common biochemical abbreviations (e.g., ATP). Other acronyms or abbreviations should either:
    - be introduced in their full form (e.g., Visualization of Polarized Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) Activity in Live Cells by Fluorescence Resonance Energy Transfer (FRET) Imaging); or
    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

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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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