International Steering Committee on Duckweed Research and Application (2013 - 15)

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Wolffiella gladiata

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1st circular for *Duckweed meeting 2015, Kyoto, Japan*

We are pleased to announce the

The Third International Conference on Duckweed Research and Applications, "Duckweed Meeting 2015, Kyoto".

We are planning to hold the 3rd ICDRA at a seminar house of Kyoto University, Japan in late June 2015. We hope this conference will be an excellent opportunity to exchange information on the latest fruits and to discuss future path of the "Duckweed community".

Kyoto was a former capital of Japan and it is a well-known international tourist city. More than a dozen of temples / shrines / castle situated in Kyoto City are registered in the UNESCO World Heritage List. You will surely enjoy the experience of Japanese culture then and now.

Kyoto city is also a landmark of the global environment preservation; the Kyoto Protocol was adopted at the COP3 conference in 1997. We hope the upcoming conference will be another landmark for the duckweed application to environmental issues globally.

We are hoping that many people will participate in the "Duckweed Meeting 2015, Kyoto".

Tokitaka Oyama, Chairman

Kyoto University, Japan

Members of the Organizing Committee of the 3rd ICDRA, 2015

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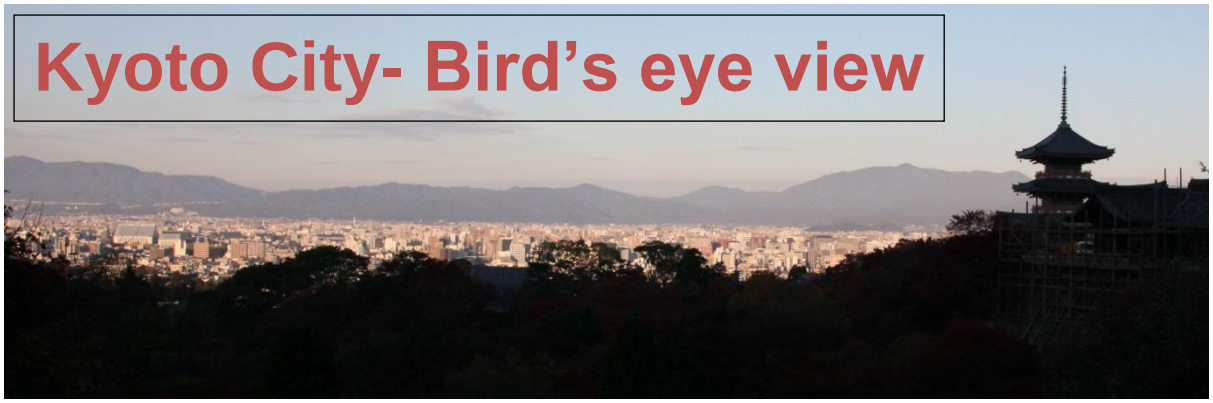
Street in Kyoto


Kyoto City- Bird’s eye view

From the data base

UPTAKE AND TOXICITY OF ARSENIC, COPPER, AND SILICON IN AZOLLA CAROLINIANA AND *LEMNA MINOR*

Rofkar, JR, Dwyer, DF, Bobak, DM

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION 16**:** 155-166 (2014)

Here we report on the analysis of two aquatic plant species, Azolla caroliniana and Lemna minor, with respect to tolerance and uptake of co-occurring arsenic, copper, and silicon for use in engineered wetlands. Plants were cultured in nutrient solution that was amended with arsenic (0 or 20M), copper (2 or 78M), and silicon (0 or 1.8mM) either singly or in combination. We hypothesized that arsenic and copper would negatively affect the uptake of metals, growth, and pigmentation and that silicon would mitigate those stresses. Tolerance was assessed by measuring growth of biomass and concentrations of chlorophyll and anthocyanins. Both plant species accumulated arsenic, copper, and silicon, L. minor generally had higher levels on a per biomass basis. Arsenic negatively impacted A. caroliniana, causing a 30% decrease in biomass production and an increase in the concentration of anthocyanin. Copper negatively impacted L. minor, causing a 60% decrease in biomass production and a 45% decrease in chlorophyll content. Silicon augmented the impact of arsenic on biomass production in A. caroliniana but mitigated the effect of copper on L. minor. Our results suggest that mixtures of plant species may be needed to maximize uptake of multiple contaminants in engineered wetlands.

Phytoremediation of Cu, Cr and Pb Mixtures by *Lemna minor*

Ucuncu, E, Tunca, E, Fikirdesici, S, Ozkan, AD, Altindag, A

BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 91**:** 600-604 **(**2013)

The present study reports the capacity of the aquatic macrophyte Lemna minor to remediate combinations of Cu(II), Pb(II) and Cr(III) from a simulated natural environment. The effect of these metal mixtures on the growth of L. minor was also investigated using growth rate and biomass inhibition calculations. L. minor was successful in removing Cr and Pb from the water, and it remained an effective remediation agent when both metals were present in the environment. However, a relatively low absorption capacity was observed for Cu, increasing concentrations of which were associated with significant decreases in growth rate. No statistically significant difference was found between the 24 h and 7 days absorption rates of Cu, Pb and Cr, suggesting that, at the concentrations tested, equilibrium occurs within 24 h of metal exposure.

Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production

van der Spiegel, M, Noordam, MY, van der Fels-Klerx, HJ

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY 12**:** 662-678 **(**2013)

Novel protein sources (like insects, algae, duckweed, and rapeseed) are expected to enter the European feed and food market as replacers for animal-derived proteins. However, food safety aspects of these novel protein sources are not well-known. The aim of this article is to review the state of the art on the safety of major novel protein sources for feed and food production, in particular insects, algae (microalgae and seaweed), duckweed, and rapeseed. Potential hazards for these protein sources are described and EU legislative requirements as regard to food and feed safety are explained. Potential hazards may include a range of contaminants, like heavy metals, mycotoxins, pesticide residues, as well as pathogens. Some safety aspects

of novel protein sources are intrinsic to the product, but many potential hazards can also be due to production methods and processing conditions. These aspects should be considered in advance during product development. European law is unclear on several issues regarding the use of novel protein sources in food and feed products. For food product applications, the most important question for food producers is whether or not the product is considered a novel food. One of the major unclarities for feed applications is whether or not products with insects are considered animal-derived products or not. Due to the unclarities in European law, it is not always clear which Regulation and maximum levels for contaminants apply. For market introduction, European legislation should be adjusted and clarified.

Short term exposure of *Lemna minor* and *Lemna gibba* to mercury, cadmium and chromium

Varga, M, Horvatic, J, Celic, A

CENTRAL EUROPEAN JOURNAL OF BIOLOGY 8**:** 1083-1093 **(**2013)

The effects of mercury (Hg), cadmium (Cd) and chromium (Cr) in concentrations ranging from 0.02 to 20 mg L-1 applied for 24 h were assessed in Lemna minor and Lemna gibba by measuring changes in protein concentration, ascorbic acid, phenolics, malondialdehyde (MDA), hydrogen peroxide (H2O2), the activity of guaiacol peroxidase (G-POX) and catalase (CAT). Ascorbic acid, phenolics, catalase and guaiacol peroxidase played a key role in the antioxidative response of L. gibba. Inadequate activity of antioxidant enzymes in the L. minor resulted in MDA and H2O2 accumulation. In both used species, Hg treatment decreased protein content and increased CAT and G-POX activity, but decreased MDA and H2O2 levels. Cadmium and chromium had opposite impacts on two used Lemna species on almost all observed parameters. Enhanced antioxidative responses of L. gibba to lower concentrations of Hg, Cd and Cr indicated greater abiotic stress tolerance than L. minor.

Alleviation of silver toxicity by calcium chloride (CaCl2) in Lemna gibba L.

Oukarroum, A, Gaudreault, MH, Pirastru, L, Popovic, R

PLANT PHYSIOLOGY AND BIOCHEMISTRY 71**:** 235-239 **(**2013)

The toxicity effects of silver (Ag) and the protective role of calcium chloride (CaCl2) was studied in Lemna gibba L. (L gibba) plants. Silver speciation showed that silver toxicity in L gibba culture medium can be attributed to free ionic Ag+ concentration. Frond abscission, intracellular reactive oxygen species (ROS) formation and intracellular uptake of Ag+ were investigated when L gibba plants were exposed to AgNO3 concentrations (0.5, 1, 5, and 10 mu M) supplemented or not by 10 mu M CaCl2. An increase in frond abscission, intracellular ROS and intracellular uptake of Ag+ were detected in L gibba plants for all tested concentrations of AgNO3 after 24 h treatment. However, addition of 10 mu M CaCl2 to the L gibba culture medium reduced the toxic effects of Ag by decreasing silver uptake into the plant and intracellular ROS formation. The results suggest that Ag-induced toxicity was attributed to Ag+ accumulation and chloride was able to protect L. gibba plants against Ag toxicity by formation of complexes with Ag and then alleviation of the metal induced oxidative stress.

Growth and photosynthesis of *Lemna minor* L. exposed to different light conditions and sucrose supplies

Vidakovic-Cifrek, Z, Soric, S, Babic, M

ACTA BOTANICA CROATICA 72**:** 211-219 **(**2013)

Duckweed (Lemna minor L.) is a model plant suitable for investigation into plant physiology, biochemistry and ecotoxicology. Depending on the type of the experiment, duckweed is cultivated on different nutrient media under various chamber conditions. In our work, duckweed was cultivated on Pirson-Seidel's nutrient solution supplemented with 5, 7.5 or 10 g L-1 sucrose under cool white (CW) or Gro-Lux (GL) light sources. When different light sources and sucrose supplies are

compared, a significant stimulative effect of GL light on duckweed grown on 7.5 and 10 g L-1 sucrose was seen to start on day 9. Considering photosynthetic performance the results showed that there were no significant differences in maximum quantum yield of PSII (F-v/F-m) after 7 and 16 days of exposure, regardless of light source and sucrose supply. Effective quantum yield of PSII (FPSII) decreased only after 16 days of exposure to 5 g L-1 sucrose under CW light. The higher growth rate and photosynthetic performance in plants exposed to GL light is a consequence of its spectral distribution resembling the action spectrum of photosynthesis. Furthermore, enhanced growth noticed in plants cultivated on higher sucrose contents (7.5 and 10 g L-1) indicated the promotive effect of sucrose in plants grown under low light conditions.

Metabolic profiling and enhanced production of phytosterols by. elicitation with methyl jasmonate and silver nitrate in whole plant cultures of *Lemna paucicostata*

Suh, HW, Hyun, SH, Kim, SH, Lee, SY, Choi, HK

PROCESS BIOCHEMISTRY 48**:** 1581-1586 **(**2013)

In this study, the effects of methyl jasmonate (MJ) and silver nitrate (SN) treatment on metabolic profiles and yields of phytosterols such as campesterol, stigmasterol, and beta-sitosterol in whole plant cultures of Lemna paucicostata were investigated using gas chromatography-mass spectrometry coupled with multivariate statistical analysis. The MJ and SN treatments retarded the growth of L paucicostata plants, while they enhanced the yields of three phytosterols, compared to control. Higher yields of phytosterols were attained at day 28 compared to day 42. Moreover, stigmasterol yield was the highest at 0.85 mg/g from day 28 plants grown under MJ + SN co-treated culture. Among the various metabolites, the levels of palmitic and stearic acids, which might participate in a defense mechanism, were higher in the MJ + SN condition than in control. To determine the optimal timing of MJ + SN addition, MJ + SN was added on days 21, 28, and 35 after inoculation. The total yield and productivity of phytosterol reached maximum levels when the MJ + SN was added at

day 35. The highest productivity of stigmasterol (6.08 mg/L) was also achieved when MJ + SN was added on day 35.

Isolation and identification of an allelopathic substance from duckweed (*Lemna minor* L.)

Bich, TTN, Ohno, O, Suenaga, K, Kato-Noguchi, H

ALLELOPATHY JOURNAL 32**:** 213-221 **(**2013)

An aqueous methanol extract of duckweed (Lemna minor L.) inhibited the growth of cress (Lepidium sativum L.). The extract was partitioned by silica gel, Diaion HP20, Sephadex LH-20 and Diaion HP20SS columns, C-18 Sep-Pak cartridges and reverse phase HPLC to isolate phytotoxic substances. The (3R)-(-)-3-hydroxy-beta-ionone was identified as the active ingredient in the extract by its spectral data. (3R)-(-)-3-hydroxy-beta-ionone inhibited growth of cress and Italian ryegrass seedlings at >= 0.1 and >= 5 mu M, respectively. (3R)-(-)-3-Hydroxy-beta-ionone inhibited 50% of cress roots and shoots growth with 0.1 mu M and 50% of Italian ryegrass root and shoot growth at 2.4 and 3.4 mu M, respectively. The presence of (3R)-(-)-3-hydroxy-beta-ionone in duckweed and its growth inhibitory activity suggested that it may contribute to the allelopathic potential of duckweed.

***Lemna minor* exposed to fluoranthene: Growth, biochemical, physiological and histochemical changes**

Zezulka, S, Kummerova, M, Babula, P, Vanova, L

AQUATIC TOXICOLOGY 140**:** 37-47 **(**2013)

Polycyclic aromatic hydrocarbons (PAHs) represent one of the major groups of organic contaminants in the aquatic environment. Duckweed (Lemna minor L) is a common aquatic plant widely used in phytotoxicity tests for xenobiotic substances. The goal of this study was to assess the growth and the physiological, biochemical

and histochemical changes in duckweed exposed for 4 and 10 days to fluoranthene (FLT, 0.1 and 1 mg L-1).
Nonsignificant changes in number of plants, biomass production, leaf area size, content of chlorophylls a and b and carotenoids and parameters of chlorophyll fluorescence recorded after 4 and 10 days of exposure to FLT were in contrast with considerable changes at biochemical and histochemical levels. Higher occurrence of reactive oxygen species (ROS) caused by an exposure to FLT after 10 days as compared to control (hydrogen peroxide elevated by 13% in the 0.1 mg L-1 and by 41% in the 1 mg L-1 FLT; superoxide anion radical by 52% and 115% respectively) reflected in an increase in the activities of antioxidant enzymes (superoxide dismutase by 3% in both treatments, catalase by 9% and 1% respectively, ascorbate peroxidase by 21% and 5% respectively, guaiacol peroxidase by 12% in the 0.1 mg L-1 FLT). Even the content of antioxidant compounds like ascorbate (by 20% in the 1 mg L-1 FLT) or total thiols (reduced forms by 15% in the 0.1 mg L-1 and 8% in the 1 mg L-1 FLT, oxidized forms by 36% in the 0.1 mg L-1 FLT) increased. Increased amount of ROS was followed by an increase in malondialdehyde content (by 33% in the 0.1 mg L-1 and 79% in the 1 mg L-1 FLT). Whereas in plants treated by the 0.1 mg L-1 FLT the contents of total proteins and phenols increased by 15% and 25%, respectively, the 1 mg L-1 FLT caused decrease of their contents by 32% and 7%. Microscopic observations of duckweed roots also confirmed the presence of ROS and related histochemical changes at the cellular and tissue levels. The assessment of phytotoxicity of organic pollutant in duckweed based only on the evaluation of growth parameters could not fully cover the irreversible changes already running at the level of biochemical processes.

A novel bioassay using root re-growth in *Lemna*

Park, A, Kim, YJ, Choi, EM, Brown, MT, Han, T

AQUATIC TOXICOLOGY 140**:** 415-424 **(**2013)

A new phytotoxicity test method based on root elongation of three Lemna species

(Lemna gibba, L. minor, and L. paucicostata) has been developed. Tests with aquatic plants have, typically, favored measurements on fronds (e.g. frond number, area, biomass) rather than on roots, due, in part, to issues associated with handling fragile roots and the time-consuming procedures of selecting roots with identical root lengths. The present method differs in that roots were excised prior to exposure with subsequent measurements on newly developed roots. Results show that there were species-specific difference in sensitivity to the five metals tested (Ag, Cd, Cr, Cu and Hg), with Ag being the most toxic (EC50 = 5.3-37.6 mu g L-1) to all three species, and Cr the least toxic for L. gibba and L. minor (1148.3 and 341.8 mu g L-1, respectively) and Cu for L paucicostata (470.4 mu g L-1). Direct comparisons were made with measurements of frond area, which were found to be less sensitive. More generally, root re-growth was shown to reflect the toxic responses of all three Lemna species to these five important metals.

The root growth bioassay differs from three internationally standardized methods (ISO, OCED and US EPA) in that it is completed in 48 h, the required volume of test solutions is only 3 ml and non-axenic plants are used. Our results show that the Lemna root method is a simple, rapid, cost-effective, sensitive and precise bioassay to assess the toxic risks of metals and has practical application for monitoring municipal and industrial waste waters where metals are common constituents.