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Response of two submersed macrophytes *Ceratophyllum demersum* and *Myriophyllum spicatum* to selenium in water

Odziv dveh potopljenih vrst makrofitov *Ceratophyllum demersum* in *Myriophyllum spicatum* na selen v vodi

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Abstract: Two submersed macrophytes (*Ceratophyllum demersum* and *Myriophyllum spicatum*) were exposed to 10 mg Se(IV) L^{-1}, later transferred to water without Se and exposed again to water containing 10 mg Se(IV) L^{-1} with the aim to observe recovery of the plants. After each transplantation trial, photochemical efficiency of photosystem II, respiratory potential and the amount of photosynthethic pigments and anthocyanins were measured. Photochemical efficiency was similar in all three trials. Electron transport system (ETS) activity increased drastically for *C. demersum* plants that were transferred from the water with Se to the water without Se, while ETS activity strongly increased in plants of *M. spicatum*, which once again grew in water containing Se. Alternation in the concentration of Se in the growth media demanded metabolic changes in studied plants. The amount of chlorophylls was higher in plants of *M. spicatum* growing in water without Se than in exposed plants, while the amount of carotenoids and anthocyanins decreased in the same species grew in water without Se.

Keywords: *Myriophyllum spicatum*, *Ceratophyllum demersum*, selenium, photochemical efficiency, respiratory potential

Izvleček: Potopljeni vrsti *Ceratophyllum demersum* in *Myriophyllum spicatum* sta bili najprej izpostavljeni koncentraciji 10 mg Se(IV) L^{-1}, kasneje smo rastline prestavili v vodo brez dodanega Se, nato pa ponovno v vodo, ki je vsebovala 10 mg Se(IV) L^{-1} z namenom, da bi ugotovili, ali se stanje rastlin, ki rastejo v vodi brez Se, izboljša. Po vsaki presaditvi in nekaj dnevih izpostavljenosti rastlin, smo izmerili fotokemično učinkovitost fotosistema II, dihalni potencial ter vsebnost fotosinteznih barvil in antocianov. Vrednosti fotokemične učinkovitosti so bile v vseh obravnavanjih podobno. Menjavanje koncentracij Se v vodi, kjer so uspevale rastline, je povzročilo spremembe v metabolizmu rastlin, kar smo izmerili s pomočjo meritev aktivnosti ETS. Vsebnost klorofilov je bila nižja pri rastlinah vrste *M. spicatum*, ki je bila izpostavljena Se, medtem ko je bila vsebnost karotenoidov in antocianov nižja pri rastlinah, ki so uspevale v vodi brez dodanega Se.

Ključne besede: *Myriophyllum spicatum*, *Ceratophyllum demersum*, selen, fotokemična učinkovitost, dihalni potencial

Introduction

Selenium (Se) is a naturally occurring trace element which is toxic at high concentrations, but it is also an essential element for many organisms (Fan et al., 2002). It is found in the Earth's crust, soils, minerals, in freshwater, seawater, and in sediments. In aquatic systems Se can be found mostly in the form of selenite and selenate (Canton and Van Derveer, 1997) and these forms are potentially toxic to aquatic organisms.

Se pollution in the environment arises from both natural and anthropogenic sources. Se is found in aquesous discharge from electric power plants, coal ash leakages, oil refinery effluents, industrial wastewater, as well as in agricultural drainage water for irrigation (Fan et al., 2002; Lemly, 2004). The addition of Se to feed stuffs and soil fertilizers is a common practice. Part of this added Se is used by animals and part is spilled or secreted and passed to the environment. Se pollution is a worldwide problem and there is a tremendous demand for cleanup of Se-contaminated water (Dhote and Dixit, 2009).

Macrophytes are aquatic plants which have been used as indicators of trace element pollution since the early seventies (Phillips, 1977). Some macrophyte species are suitable for wastewater treatment because they have a tremendous capacity for absorbing nutrients and other substances from the water (Boyd, 1970) and hence reduce the pollution. Some aquatic plants can take up trace elements through their roots whereas in submersed plants such are *Myriophyllum alternifolium*, *Vallisneria spiralis*, *Chara carolina* and *Veronica aquatica* (Delmail et al., 2011; Rai et al., 1995; Robinson et al., 2006), leaves as well as roots take part in uptake. In previous studies it was evidenced that *Myriophyllum spicatum* and *Ceratophyllum demersum* took up a large amount of trace elements (Rai et al., 1995; Robinson et al., 2006; Mechora et al., 2011).

The purpose of our study was to investigate the difference in response of *M. spicatum* L. and *C. demersum* L. growing in water with Se(IV) and water without Se. We also want to observe the recovery of the plants. To reach these aims, we measured physiological and biochemical parameters of plants, namely the photochemical efficiency of PSII, electron transport system (ETS) activity and content of photosynthetic pigments.

Materials and Methods

Plants and growth conditions

Experiments were conducted under natural conditions at Ljubljana, Slovenia. *Myriophyllum spicatum* was obtained from Lake Bohinj (547 m asl, 46º17´ N, 13º54´ E, Slovenia) and planted on 15 April 2011, while *Ceratophyllum demersum* was obtained from a pond in the Botanical Garden (Ljubljana: 320 m asl, 46º35´ N, 14º55´ E, Slovenia) and placed in containers on 18 April 2011. Both species were placed in two separate containers of size 120 cm x 52 cm x 54 cm, containing 160 L of tap water and layer of soil and sand.

After two weeks of plant acclimatization, natrium selenite (Na_2SeO_3) was added to the experimental containers. One container had water without Se, while the second contained 10 mg Se L^{-1}. During the experiment the concentration of Se in water was measured and maintained at the desired levels.

After 5 days of an exposure to Se, we measured physiological and biochemical parameters (photochemical efficiency of photosystem II, respiratory potential and the amount of photosynthetic pigments and anthocyanins) and then transferred the plants to the container with water without added Se. After 10 days of exposure, the selected parameters were measured again and then plants were transferred back to 10 mg Se L^{-1} solution. After 5 days of exposure selected parameters were measured again in plants, transferred again to water containing 10 mg Se L^{-1}.

Photochemical efficiency

Chlorophyll fluorescence was measured *in situ* on ten vital plants from each container using a fluorometer (PAM 2100 Chlorophyll Fluorometer, Heinz Walz GmbH, Germany). The potential quantum yield was evaluated in terms of the ratio F_v/F_m. Measurements of minimal (F_0) and maximal (F_m) chlorophyll fluorescence were made after 10 min of darkness, provided by dark-adaptation clips. Fluorescence was excited with a

saturating beam of "white light" (PPFD = 8 000 μmol m^{-2} s^{-1}, 0.8 s).

Electron transport system (ETS) activity

The respiratory potential of mitochondria was measured as terminal electron transport system (ETS) activity in plants (Packard, 1971). 0.2–0.4 g of leaves from four plants from container with added Se and without added Se were homogenized using ice-cold homogenization buffer and sonication with an ultrasound homogenizer (40W, 4710, Cole-Parmer, Vernon Hills, IL, USA). The homogenate was then centrifuged (10000 rpm, for 4 min, 0 ºC) in a top-refrigerated ultracentrifuge (Sigma 2–16 PK, Germany). We added 1.5 mL of substrate solution and 0.5 mL of iodo-nitrotetrazolium chloride (INT) to triplicates of the supernatant (0.5 mL), and incubated at 20 ºC for 40 min. INT instead of oxygen was reduced to formazan during incubation. After stopping the reaction with formaldehyde and phosphoric acid (1:1), the formazan absorption at 490 nm was measured. ETS activity was determined as the rate of tetrazolium dye reduction and conversion to oxygen equivalents (Kenner and Ahmed, 1975).

Photosynthetic pigments

For content of chlorophylls *a* and *b* and carotenoids, leaves of four plants from container with added Se and without Se were selected. Chlorophylls and carotenoids were extracted with 90 % acetone. Extracts were centrifuged in a refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany) at 4000 rpm for 4 min at 4 ºC. Absorbance was measured with a UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA) at 470 nm, 644 nm and 662 nm. The amounts of pigments were determined as described by Lichtenthaler and Buschmann (2001a and 2001b). The total anthocyanin content was measured as described by Drumm and Mohr (1978).

Statistical analysis

The significance of the difference between mean values was determined by the analysis of variance with LSD test. Differences at $p < 0.05$ were considered as statistically significant.

Results and discussion

Measurements of physiological and biochemical parameters can show the status of the plants. Photochemical efficiency, respiratory potential measured as electron transport system (ETS) activity as well as photosynthetic pigments can be a good indicator of stress. The values of F_v/F_m around 0.8 indicate that plants are in good condition (Schreiber et al., 1995). In plants, exposed to water with 10 mg Se L^{-1}, the values of photochemical efficiency were around 0.36 for *C. demersum* and 0.48 for *M. spicatum* (Fig. 1). These values indicated that Se exposed plants were under stress. Photochemical efficiency in plants slightly increased, when plants were transferred to water without Se, but there was no statistically significant difference (Fig. 1). On the other hand F_v/F_m was higher in *M. spicatum* and *C. demersum*, exposed to 10 mg Se(VI) L^{-1} comparing to untreated plants (Mechora et al., 2011). After transferring the plants to Se solution the values of F_v/F_m slightly decreased again, the values being 0.33 in *C. demersum* and 0.46 in *M. spicatum* (Fig. 1).

ETS activity was the lowest in plants exposed to Se at the beginning of the experiment (Fig. 1). ETS activity increased drastically in *C. demersum* that were transferred to the water without added Se. In the case of *M. spicatum* ETS activity strongly increased in plants, which once again grew in water containing Se. Any changes in environmental parameters request metabolic adaptation (Larcher, 2003). This is in concordance to our results in the transplantation experiment.

The content of chlorophyll *a* in *M. spicatum* increased, when plants were transplanted into water without Se, while the content of chlorophyll *b* decreased (Table 1), but there was no statistically significant difference in later. In other study, the addition of Se(VI) had no effect on the amount of chlorophylls in *M. spicatum* (Mechora et al., 2011), while Cd lowered the amount of chlorophylls in *M. alternifolium* (Delmail et al., 2011) and *M. spicatum* (Sivaci et al., 2004). In *C. demersum* the amount of chlorophylls was the lowest in water without Se (Table 1), however the results were not statistically significant. This could suggest that Se did not affect the synthesis of chlorophylls in *C. demersum*.

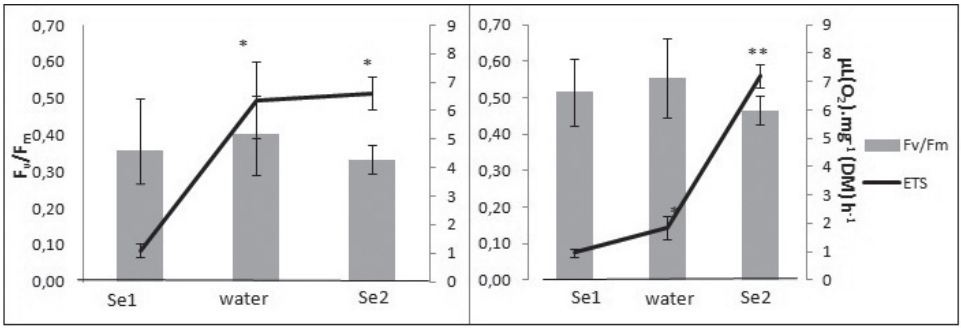

Figure 1: Photochemical efficiency and ETS activity in *Ceratophyllum demersum* (left) and *Myriophyllum spicatum* (right). Each parameter was tested separately for each species. Results with * were statistically different from the others at $p < 0.05$. Se1 – exposed to 10 mg Se L^{-1} for the first time; Se2 – exposed to 10 mg Se L^{-1} for the second time.

Slika 1: Fotokemična učinkovitost in aktivnost ETS pri vrsti *Ceratophyllum demersum* (levo) in *Myriophyllum spicatum* (desno). Vsak parameter je bil testiran posebej za vsako vrsto. Rezultati označen z * je statistično značilno različen od drugih pri $p < 0.05$. Se1 – izpostavljene prvič 10 mg Se L^{-1}; Se2 – izpostavljene drugič 10 mg Se L^{-1}.

The content of carotenoids and anthocyanins in *M. spicatum* and in *C. demersum*, grew in water without Se (Table 1) was lower comparing to plants, grew in Se solution, but there was no statistically significant difference in later species. On the contrary, a negative effect of Cd on the amount of carotenoids was observed in *M. alternifolium* (Delmail et al., 2011) and *M. spicatum* (Sivaci et al., 2004). Carotenoids and anthocyanins can start to accumulate in plants, when they are in stress (Winkel-Shirley, 2002) that is in line with the present results (Table 1).

Table 1: The amount of pigments in macrophytes growing in water and Se solution; n = 4, RU – relative units.
Tabela 1: Vsebnost barvil v makrofitih, ki so rastli v vodi brez in z dodanim Se; n = 4, RU – relativne enote.

	Ceratophyllum demersum			***Myriophyllum spicatum***		
	10 mg L^{-1}	water	10 mg L^{-1}	10 mg L^{-1}	water	10 mg L^{-1}
Chlorophyll *a* (mg g^{-1} DM)	0.82±0.12	0.44±0.13	0.84±0.22	0.52±0.26	1.12±0.13*	0.81±0.17
Chlorophyll *b* (mg g^{-1} DM)	0.59±0.12	0.27±0.11	0.64±0.40	0.83±0.45	0.54±0.08	0.33±0.10
Carotenoids (mg g^{-1} DM)	0.44±0.05	0.20±0.07	0.33±0.04	0.90±0.21*	0.37±0.06	0.38±0.03
Anthocyanins (RU g^{-1} DM)	97±23	37±15	220±45	160±10*	59±36	130±71*

Each parameter was tested separately for each species. Results with * were statistically different from the others at $p < 0.05$.

Conclusions

Photochemical efficiency was similar in macrophytes exposed to 10 mg Se(IV) L^{-1}, in water without Se and in water, containing 10 mg Se(IV) L^{-1} once again. Alternation in the concentration of Se in the water in transplantation experiment demanded metabolic changes in studied plants, which were evidenced by the measurement of ETS activity. The amount of chlorophylls was higher in plants of *M. spicatum* growing in water without Se in comparison to plants growing in water with Se, while the amount of carotenoids and anthocyanins decreased in water without Se for this speices. The recovery of the plants, grew in water, was not observed.

Zaključki

Makrofiti so bili izpostavljeni 10 mg Se(IV) L^{-1}, kasneje smo jih prestavili v vodo brez dodanega Se, nato pa ponovno v vodo, ki je vsebovala 10 mg Se(IV) L^{-1}. Vrednosti fotokemične učinkovitosti so bile v vseh obravnavanjih podobne. Menjavanje koncentracij Se v mediju, kjer so rastline rastle, je povzročilo spremembe v metabolizmu rastlin, kar se je pokazalo pri meritvah aktivnosti ETS. Vsebnost klorofilov je bila nižja v vrsti *M. spicatum*, ki je bila izpostavljena Se v primerjavi z rastlinami, ki so rastle v vodi brez dodanega Se, medtem ko je bila vsebnost karotenoidov in antocianov nižja pri rastlinah, ki so uspevale v vodi brez dodanega Se. Pri rastlinah v vodi brez dodanega Se ni bilo opaznega izboljšanja stanja rastlin.

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