DOI: 10.2429/proc.2012.6(2)067 2012;6(2)

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# DIFFERENTIAL SENSITIVITY OF THE *Lemnaceae* SPECIES TO CHROMIUM AND ZINC

## WRAŻLIWOŚĆ RODZINY *Lemnaceae* NA CHROM I CYNK

**Abstract:** The aim of study was to optimize alternative acute toxicity tests involving toxic compounds of Cr(VI) and Zn(II) and two representatives of the *Lemnaceae* species (*Lemna gibba* and *Spirodela polyrhiza*) as testing organisms and to compare their relative sensitivity with *Lemna minor* and *Daphnia magna*. The most sensitive organism to both metals was *D. magna*. The highest duckweed sensitivity to Cr(VI) and Zn(II) was determined for *S. polyrhiza* and *L. gibba*, respectively. The compound of Cr(VI) provided significantly higher toxicity effect towards the testing organisms than Zn(II). *L. gibba* proved the most suitability for assessment of environmental quality due to the highest duckweed sensitivity. In contrast, *L. minor* and *S. polyrhiza* showed the lowest sensitivity to Cr(VI) and Zn(II), respectively. It was probably caused by their antioxidant ability, and thus they may be the most effective for removal of metals from water environment.

**Keywords:** ecotoxicological test, chromium, zinc, the *Lemnaceae* family, acute toxicity test, growth inhibition

An environmental pollution by various pollutants is a serious problem with harmful impacts on living organisms (*eg* gene mutation, cancer, influencing of growth, development, and reproduction) [1, 2]. Inorganic compounds containing arsenic, cadmium, chromium, copper, mercury, zinc, or lead that are easily accumulated in soil or living organisms are considered to be the most detrimental elements to the environment [2-4]. The aquatic plants from the *Lemnaceae* family are able to accumulate heavy metals [5, 6]. The danger is that they serve as feed for fish and waterfowl and thus may be a potential risk for humans [2]. On the other hand, they can help in removing excess of toxic metals from surface water [6-8]. The aim of study was to optimize alternative acute toxicity tests involving toxic compounds of Cr(VI) and Zn(II) and two *Lemnaceae* species (*Lemna gibba* and *Spirodela polyrhiza*) as testing organisms, and to compare their relative sensitivity with *Lemna minor* and *Daphnia magna*.

## Experimental

All chemicals used were of analytical grade. Stock solutions and culture media were prepared according to the ISO 20079 [9] and ISO 6341 protocols [10]. Solutions of $K_2Cr_2O_7$ (200 mg/dm$^3$) and $ZnSO_4 \cdot 7H_2O$ (100 mg/ dm$^3$) were used as stock standards.

Alternative growth inhibition tests and the standard growth inhibition test with *L. minor* were performed according to the biotest based on the ISO 20079 protocol [9]. Tested duckweed culture media contained 0÷140, 0÷50, 0÷30 mg/cm$^3$ $K_2Cr_2O_7$, or 0÷30, 0÷10, and 0÷40 mg/cm$^3$ $ZnSO_4 \cdot 7H_2O$ for *L. minor*, *L. gibba*, and *S. polyrhiza,*

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respectively. Tested species of duckweeds were taken from billabong river Orlice. To the tests were chosen completely healthy duckweed plants with 2-4 fronds after laboratory recultivation. Total number of fronds in 100 $cm^3$ solutions was 20 or 10 for *L. minor* and *L. gibba*, or *S. polyrhiza*, respectively. All solutions were covered by transparent film and placed into thermostat at 24°C with permanent light. Status of plants and number of duckweed fronds were controlled for all tested concentrations during 14 days. Growth inhibition percentages ($I$) were calculated according to $I = (C - S)/C \cdot 100\%$, where $C$ and $S$ represent the number of live fronds in control and sample, respectively. The half maximal inhibitory concentrations (IC50), which mean 50% duckweed growth inhibition, were determined using regression equations from the dependences of growth inhibition on concentration of toxic compound.

The standard acute toxicity test with *Daphnia magna* was carried out according to the biotest based on the ISO 6341 protocol [10]. Tested daphnia cultivation media contained 0÷2 mg/$cm^3$ $K_2Cr_2O_7$ and 0÷10 mg/$cm^3$ $ZnSO_4 \cdot 7H_2O$. Only moving daphnids of age in 24 hours from prebreeding were used for the tests and their total number reached 10 in 100 $cm^3$ (max. 1 daphnia/5 $cm^3$). Death loss in control solution was lower than 10%. All solutions were placed into thermostat at 20°C with the ratio of light cycle 16 : 8 (light : darkness) without aeration and feed. Numbers of immobilized daphnids were determined after 24 and 48 hours for all tested concentrations. Mortality percentages ($M$) was calculated according to $M = (C - S)/C \cdot 100\%$, where $C$ and $S$ represent the number of immobilized daphnids in control and sample, respectively. The half maximal effective concentrations (EC50), which refer to 50% inhibition of their mobility, were determined using regression equations from the dependences of death loss on concentration of toxic compound.

## Results and discussion

The presented study deals with the comparison of relative sensitivity of three aquatic plants from the *Lemnaceae* family to compounds comprising hexavalent chromium and bivalent zinc as potential toxic metals. Common duckweed (*L. minor*) is a worldwide species which is commercially used in ecotoxicological laboratories and research. Figure 1 shows the deleterious impact of zinc sulfate on the growth of *L. minor*. Fat duckweed (*L. gibba*) and greater duckweed (*S. polyrhiza*) were selected as representatives of duckweed species naturally occurring in Czech Republic and their potential sensitivity was compared with *L. minor* and *D. magna*.

Figure 2 displays the assessment of growth inhibition of *L. gibba* and *S. polyrhiza* induced by potassium dichromate and zinc sulfate. All determined IC50 and EC50 are stated in Table 1. Comparison of the relative sensitivity of three duckweed species, and *Daphnia magna* is presented in Figure 3. The chromium and zinc sensitivity decrease in the order: *D. magna* > *S. polyrhiza* > *L. gibba* > *L. minor* and *D. magna* > *L. gibba* > *L. minor* > *S. polyrhiza*, respectively.

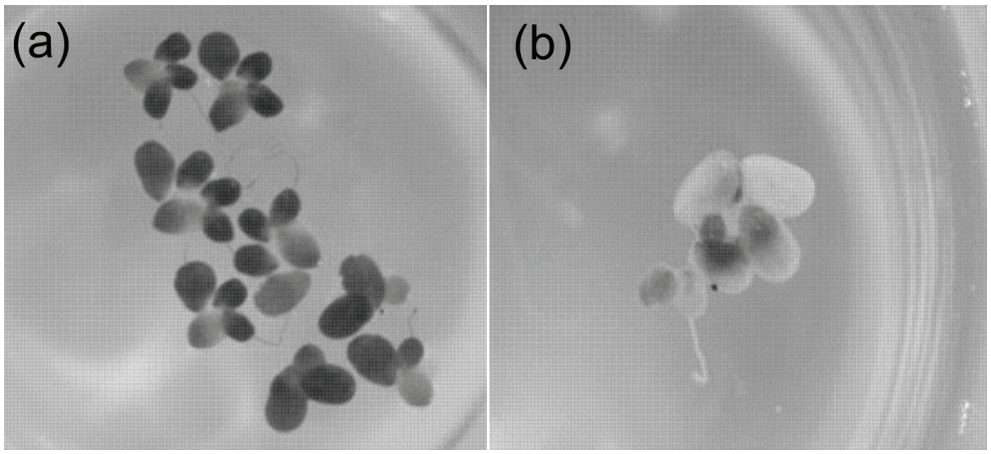


Fig. 1. Lesser duckweed (*Lemna minor*) in the absence (a) and presence (b) of $ZnSO_4 \cdot 7H_2O$ (10 mg/dm$^3$) after 14 days at 24°C

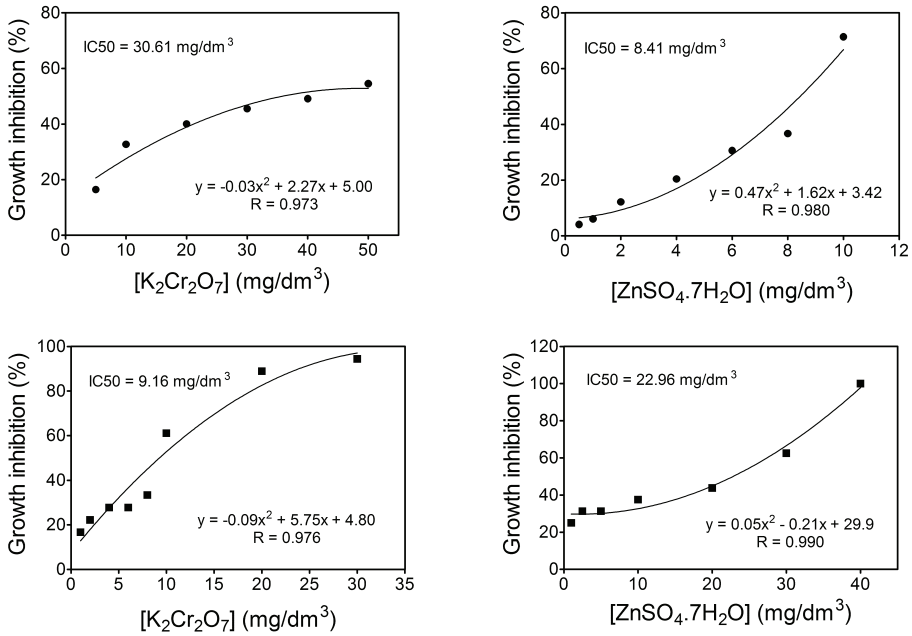


Fig. 2. Growth inhibition of *Lemna gibba* (●) and *Spirodela polyrhiza* (■) by $K_2Cr_2O_7$ and $ZnSO_4 \cdot 7H_2O$ solutions after 14 days at 24°C

The obtained results showed that the most sensitive organism to both studied compounds was aquatic arthropod *D. magna*. The highest duckweed sensitivity to Cr(VI)

was determined for *S. polyrhiza*. It was more than six or nine times lower in the comparison with *D. magna* after 24 or 48 h, respectively. *L. gibba* showed more than three times and *L. minor* even seven times lower sensitivity to potassium dichromate than *S. polyrhiza*. The highest duckweed sensitivity to Zn(II) was noticed for *L. gibba*. Its IC50 value was close or half to the EC50 values of *D. magna* after 24 h or 48 h, respectively. *L. minor* showed a slightly lower and *S. polyrhiza* less than three times lower sensitivity to zinc sulfate than *L. gibba*. The compound of hexavalent chromium possessed significantly higher toxic impact towards the testing organisms. The obtained results showed that *L. gibba* is the most suitable duckweed for assessment of environmental quality due to its highest sensitivity towards the tested compounds.

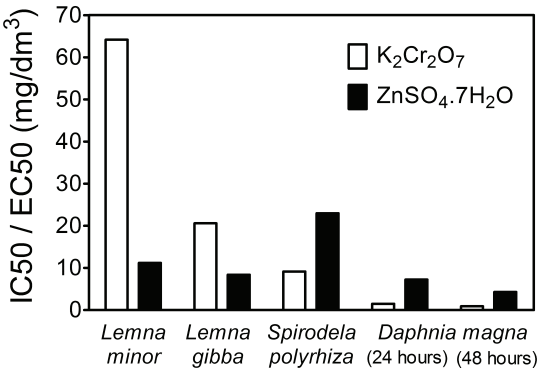


Fig. 3. Sensitivity of the tested *Lemnaceae* species and *Daphnia magna*

Table 1

Growth inhibition of the tested *Lemnaceae* species after 14 days and death loss of *Daphnia magna* caused by $ZnSO_4 \cdot 7H_2O$ and $K_2Cr_2O_7$

| Organism | IC50 [mg/dm$^3$] | | | |
|---|---|---|---|---|
| | **$K_2Cr_2O_7$** | **$ZnSO_4 \cdot 7H_2O$** | **Cr[a]** | **Zn[a]** |
| *Lemna minor* | 64.2 | 11.2 | 22.7 | 2.55 |
| *Lemna gibba* | 30.6 | 8.41 | 10.8 | 1.91 |
| *Spirodela polyrhiza* | 9.16 | 23.0 | 3.24 | 5.06 |
| | **EC50 [mg/dm$^3$]** | | | |
| | **$K_2Cr_2O_7$** | **$ZnSO_4 \cdot 7H_2O$** | **Cr[a]** | **Zn[a]** |
| *Daphnia magna* (24 hours) | 1.51 | 7.24 | 0.54 | 1.64 |
| *Daphnia magna* (48 hours) | 0.92 | 4.30 | 0.32 | 0.98 |

[a] Conversion to IC50 or EC50 related to toxic metal

Also Lahive et al [11] observed that *L. gibba* is more sensitive to zinc sulfate than *L. minor*. In contrast, no significant difference in sensitivity towards potassium dichromate between *L. minor* and *L. gibba* was noticed by Cowgill et al [12] and Henke et al [13] observed no obvious induction of frond abscission in *L. minor* caused by Cr(VI) and Zn(II). It was described that the *Lemnaceae* species are able to accumulate heavy metals and some organic pollutants [12, 14]. Consequently they can be involved in food chain and threatened

living organisms including humans [2]. On the other hand, the duckweed species may be used for efficient elimination of toxic pollutants and thus may ultimately serve as phytoremediation agents in the natural environment [6-8, 14]. It was showed that *L. gibba* was able to remove metals such zinc, copper, or cadmium from the aqueous medium [8]. Our obtained data suggest that *L. minor* and *S. polyrhiza*, which showed the lowest chromium and zinc sensitivity, respectively, may be the most effective for removal of these metals from water environment. The decrease of duckweed sensitivity to toxic compounds is probably related to their antioxidant ability which saves the plants from cells damage caused by production of reactive oxygen species during the oxidative stress. Uruc-Parlak and Demirezen Yilmaz [15] proposed that *L. minor*, *L. gibba*, and *S. polyrhiza* are supplied with an efficient antioxidant mechanism against zinc induced oxidative stress. Radic et al [16] demonstrated that *L. minor* has a potential for phytoremediation of water bodies polluted by zinc and aluminum due to its high bioaccumulation potential and tolerance *via* increased antioxidant capacity.

## Conclusions

The obtained results showed that the most sensitive organism to both studied compounds was *D. magna*. The hexavalent chromium provided significantly higher toxicity effect towards the testing plants than bivalent zinc. The highest sensitivity of duckweed species to Cr(VI) and Zn(II) was determined for *S. polyrhiza* and *L. gibba*, respectively. *L. gibba* proved the most suitability for assessment of environmental quality due to its highest sensitivity from the tested duckweeds. On the other hand, *L. minor* and *S. polyrhiza* showed the lowest sensitivity to Cr(VI) and Zn(II), respectively, and thus may be the most effective for removal these metals from water environment. It is probably related to their efficient antioxidant ability.

## Acknowledgements

The presented study was supported by the Specific research 2011 of the University of Hradec Králové and by the grant PL.3.22/2.3.00/11.02553.

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## WRAŻLIWOŚĆ RODZINY *Lemnaceae* NA CHROM I CYNK

**Abstrakt:** Celem pracy była optymalizacja alternatywnych testów ostrej toksyczności z udziałem toksycznych związków Cr(VI) i Zn(II) i dwóch przedstawicieli gatunku Lemnaceae (*Lemna gibba* i *Spirodela polyrhiza*) jako organizmów testowych i porównanie ich względnej czułości w stosunku do *Lemna minor* i *Daphnia magna*. Organizmem najbardziej wrażliwym na oba metale była *D. magna*. Największą wrażliwość na Cr(VI) i Zn(II) stwierdzono odpowiednio dla *L. gibba* i *S. polyrhiza*. Związek chromu(VI) powodował silniejszy efekt toksyczny w organizmach testowych niż Zn(II). Ze względu na najwyższą czułość *L. gibba* jest najlepszym biomonitorem do oceny jakości środowiska naturalnego. Najmniejszą wrażliwość na Cr(VI) i Zn(II) wykazały odpowiednio *L. minor* i *S. polyrhiza*. Jest to prawdopodobnie spowodowane ich zdolnościami antyoksydacyjnymi i dlatego mogą być one najbardziej skuteczne w usuwaniu metali ze środowiska wodnego.

**Słowa kluczowe:** badania ekotoksykologiczne, chrom, cynk, rodzina *Lemnaceae*, badanie ostrej toksyczności, zahamowanie wzrostu