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Effects of Oil Industry High Density Brines in Miniaturized Algal Growth Bioassay and Lemna Test

By

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Summary

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Aquatic primary producers have been frequently used for toxicity evaluation of many pollutants. Duckweeds and algae have different levels of complexity and could complement each other in phytotoxicity bioassays. Saturated water solutions of $CaCl_2$, $CaBr_2$ and their 1:1 mixture are commonly used for pressure control in oil wells. The effects of these solutions on the green alga *Chlorella kessleri* and on the macrophyte *Lemna minor* were studied. The 0.5%, 1.0% and 1.5% (v/v) concentrations of all tested solutions stimulated the growth of *C. kessleri* during later phase of experiment. $CaBr_2$ in 2.0% (v/v) concentration inhibited the growth during the whole experiment, while $CaCl_2$ and 1:1 mixture just at the beginning. In Lemna test 1.0% and 1.5% (v/v) concentrations of all tested solutions also caused the stimulation of growth at the later phase of experiment, while all three solutions in 2.0% concentration showed the inhibition of *L. minor* growth during the whole experiment.

Introduction

Aquatic plants represented by a variety of algal and macrophytic species are increasingly used in phytotoxicity tests. The unicellular green alga *Chlorella kessleri* has been recently used in miniaturized algal growth bioassay to determine

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the nutrients limiting algal growth, but it is also useful for toxicity testing (LUKAVSKY 1994). Duckweed *Lemna minor* is a small, widely spread vascular floating macrophyte that grows rapidly and reproduces vegetatively. Easiness of culture and possibility of manipulation in aseptic laboratory conditions make it suitable test organism for toxicity evaluation of many pollutants (LEWIS 1995, WANG 1990, 1991).

Calcium chloride and calcium bromide aqueous solutions (densities 1300 g dm^{-3} and 1610 g dm^{-3}, respectively) as well as their 1:1 mixture are industrial chemicals commonly used as high density brines, called also "heavy brines" for pressure control in oil wells. These solutions could be accidentally released and pollute water ecosystems nearby oil wells.

The purpose of our study was to determine the effects of $CaCl_2$, $CaBr_2$ and their 1:1 mixture on growth of *Chlorella kessleri* and *Lemna minor*.

Materials and Methods

Saturated water solutions of $CaCl_2$ and $CaBr_2$ (concentrations 481.3 g dm^{-3} and 1065.9 g dm^{-3}, respectively) and their 1:1 mixture were used as stock solutions. The solutions were of technical grade. By atomic absorption spectroscopy (AAS) certain amounts of Mg^{2+} and Zn^{2+} ions were found and amounts of heavy metals Cd, Cr, Ni, V, Fe and Co were not detectable.

An unicellular green alga *Chlorella kessleri* Fott et Novák, strain LARG/1 was supplied by Cult. Coll. Autotroph. Org., Třeboň, Czech Republic. Alga was exposed to continuos overhead light, intensity 138 $\mu E\ m^{-2}\ s^{-1}$ and temp. 20 °C.

Lemna minor L. was collected in Botanical garden of Faculty of Science, University of Zagreb. Plants were sterilised according to KRAJNČIČ & DEVIDE 1980 and maintained as stock cultures under 16 hours of light (80 $\mu E\ m^{-2}\ s^{-1}$) at 24 ± 2 °C on the Pirson and Seidel's medium (PIRSON & SEIDEL 1950).

Sample concentrations (0.5%, 1.0%, 1.5% and 2.0%, v/v) were prepared by diluting stock solutions: for miniaturized algal bioassay (Chlorella test) with modified Pratt's nutrient solution and for Lemna test with modified Hoagland's nutrient solution.

Immunological plates (FB, 9×12 cm, 96 wells of 0.25 ml) were used for Chlorella test. Twelve replicates were filled with 150 µl of the same sample and plates were sterilized 2 hours, under an UV light. In marginal wells was only distilled water. Testing alga was diluted to 400000 cells/ml and 50 µl of inoculum was added in wells. Absorbance at 750 nm was measured weekly using an Uniscan II spectrophotometer. Growth was estimated by evaluation of dry weights according to formula: $DW = 3.31 + 179.45 \cdot A_{750} + 617.45 \cdot A^2_{750}$ (mg/dm^3).

For Lemna test healthy colonies with 2-3 fronds were transferred from stock cultures into the Erlenmeyer flasks containing 60 ml of sterilised samples. Each treatment and the control were prepared in eight replicates. Frond number was counted during 2-weeks test period on days 0, 3, 5, 8, 10, 12 and 14. Growth was estimated as change in the frond number according to formula:

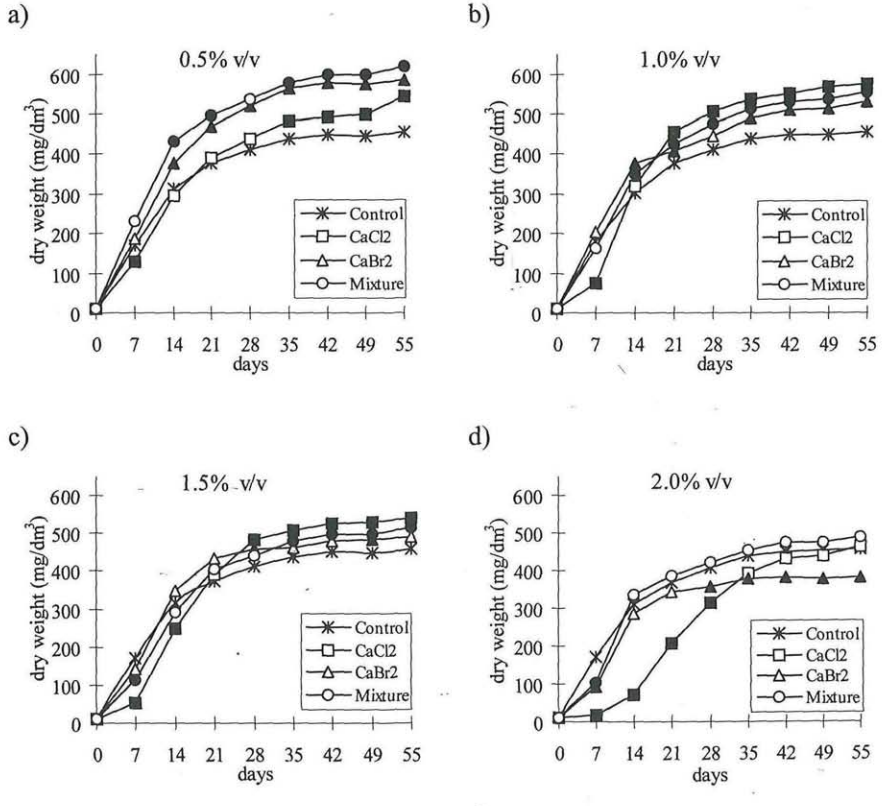
$$\frac{\text{no. of fronds at day n - no. of fronds at day 0}}{\text{no. of fronds at day 0}} \qquad n = 3, 5, 8, 10, 12, 14$$

The results were given as mean of all replicates and compared to control by Student's t-test.

Results and Discussion

It is well known that some salts, even heavy metals, when present in small amounts could cause a stimulation of plant growth but in higher amounts are toxic (LUCKEY & al. 1975). In our experiment lower concentrations of tested chemicals (0.5%, 1.0% and 1.5% v/v) caused the stimulation of growth in both test systems during the later phase of experiment, although in some cases at the beginning the inhibition was noticed (Fig 1). It confirms that plants as highly adaptive organisms could overcome certain extent of increased ion concentration and then achieve normal, even stimulative growth rate (SUBHADRA & al. 1991).

The highest concentration tested (2.0% v/v) inhibited *L. minor* growth during the whole experiment (Fig. 1, h) while *C. kessleri* just at the beginning (Fig. 1, d). The inhibition of growth could be the consequence of non-specific osmotic effect or specific toxicity of certain ions (SERRANO & GAXIOLA 1994). In tested solutions toxicity could be due to Ca^{2+}, Br^- or Cl^- ions because inorganic impurities can not have significant effect on growth in used dilutions. Our recent experiment with chemicals of analytical grade confirms that, because the effect of



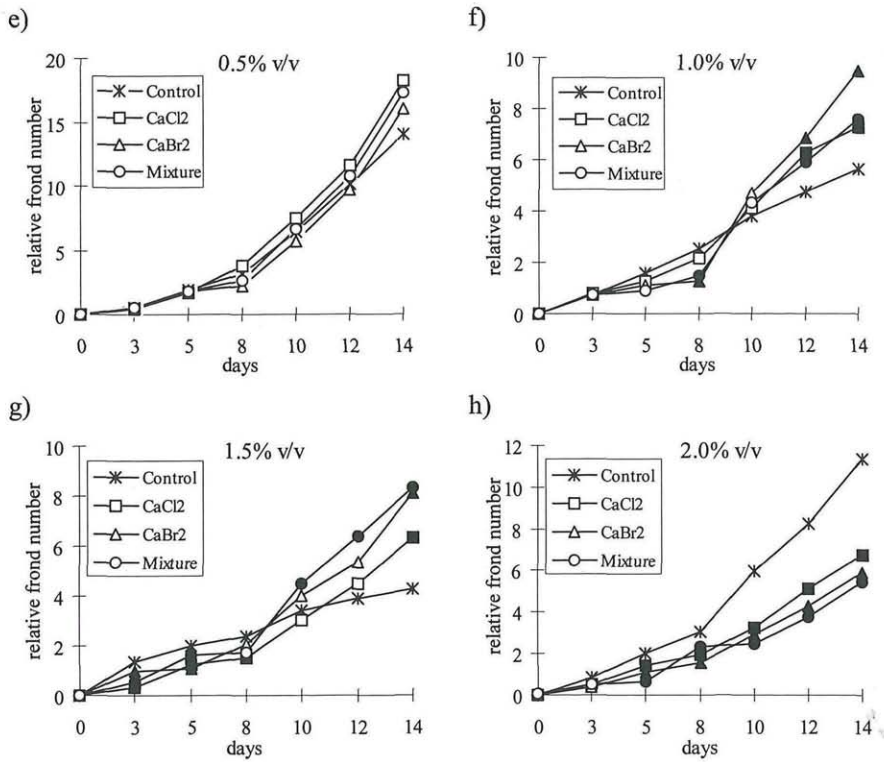


Fig. 1. The effect of $CaCl_2$ (□), $CaBr_2$ (Δ) and their 1 : 1 mixture (○) on growth of *Chlorella kessleri* (a, b, c, d) and *Lemna minor* (e, f, g, h) in comparison with the control (✳). Significantly less or higher growth than the control ($P < 0.05$ by Student's t-test) is marked with black symbols.

analytical and technical chemicals on growth was very similar (results not shown). It is possible that bromide is more toxic for *C. kessleri* because among all three samples in 2.0% only $CaBr_2$ inhibited the growth during the whole experiment.

Also it could be noticed that samples in 0.5% (v/v) did not have the same significant effect on *L. minor* growth as on *C. kessleri* so alga seems to be more sensitive to smaller amounts of these salts.

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