

# Combined assessment of salinity and heavy metal stress using two ciliate species



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## MATERIALS AND METHODS

### Ciliate cultures:

*Paramecium dubosqui*

*Paramecium nephridiatum*

Each of the species was kept as two strains adapted to two different salinities - 0.5 and 5‰, required salinity was obtained by adding a solution of standardized artificial sea salt to standardized lettuce media with *Enterobacter sp.* as food organism (1).

### Toxicants:

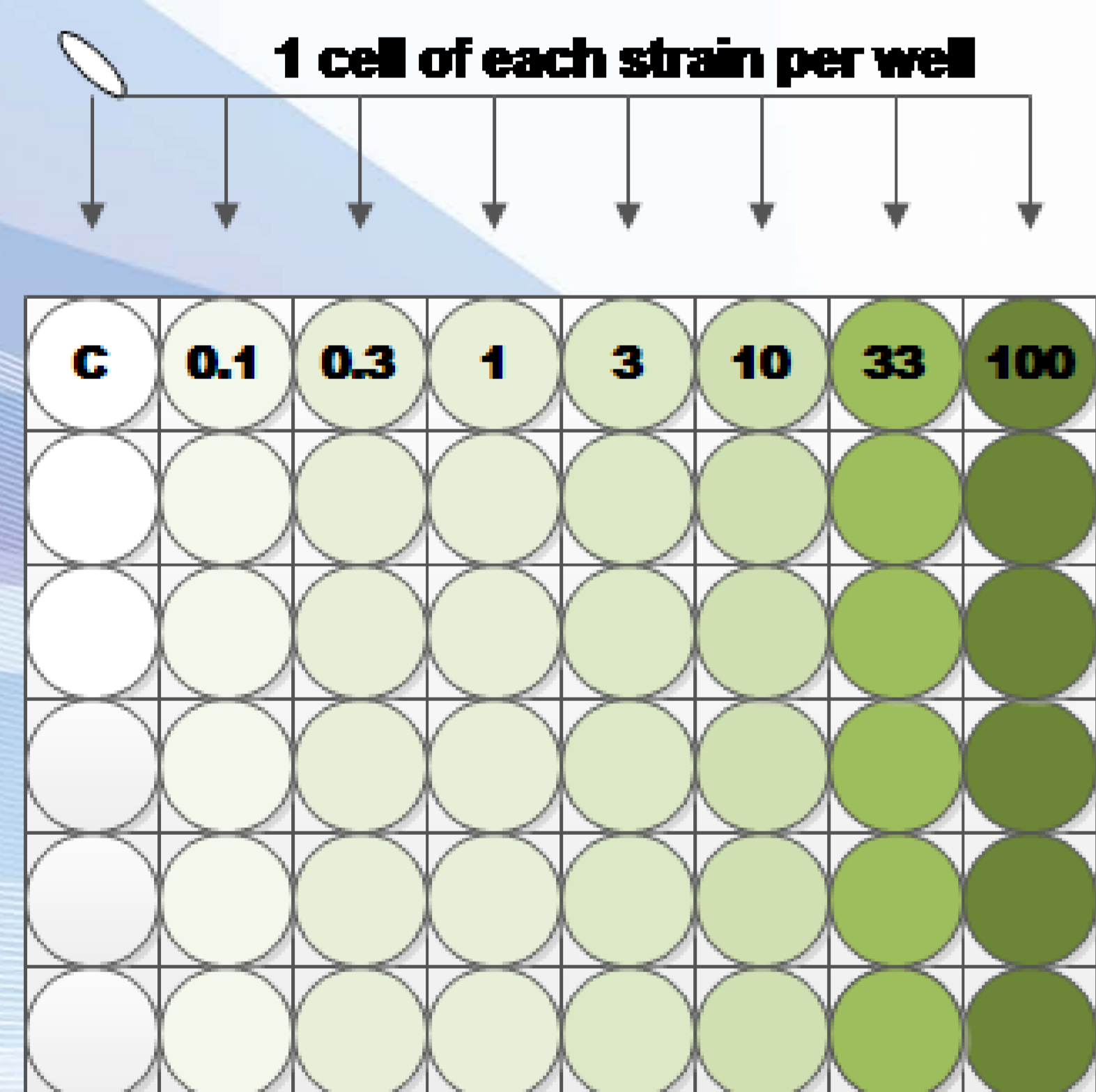
K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>

CuCl<sub>2</sub>·2H<sub>2</sub>O

For test, stock solutions of both metals were added to media to obtain following concentrations - control (no metal added), 0.1, 0.3, 1, 3, 10, 11, 100 mg/L (each species tested at two salinities - 0.5 and 5‰)

Tests were performed in 48-well microplates, 6 wells per each treatment or control. The test started with one *Paramecium* cell per each culture well. Reproduction (cell division) was monitored during following 3 days by recording the number of live cells, where cell motility was assessed as a criterion of viability (2).

Numbers in circles – concentrations of toxicants (mg/L) in the media of appropriate salinity (0.5 and 5‰)



Calculation of number of live cells in each well  
(motility used as vitality criteria)

## REFERENCES

- 1 Sonneborn TM. 1950. Methods in the general biology and genetics of *Paramecium aurelia*. *Journal of Experimental Zoology* 113:87-147
- 2 MaMadoni P, Romeo M. 2006. Acute toxicity of heavy metals towards freshwater ciliated protists. *Environmental Pollution* 141:1-7.

## INTRODUCTION

The main idea of present study was to develop a biological model to assess the combined effects of salinity and chemical toxicity in the areas with gradients of salinity (estuaries, fjords, brackish water bodies, etc.) using the euryhaline ciliates.

Biological assessment of mentioned areas is complicated because traditionally used organisms are either freshwater or marine, and do not survive or reproduce in higher or lower salinities. Use of euryhaline protozoans has several advantages such as easy adaptation to a range of salinities, small size, easy breeding and fast growth making them good and easy to handle laboratory model.

## RESULTS

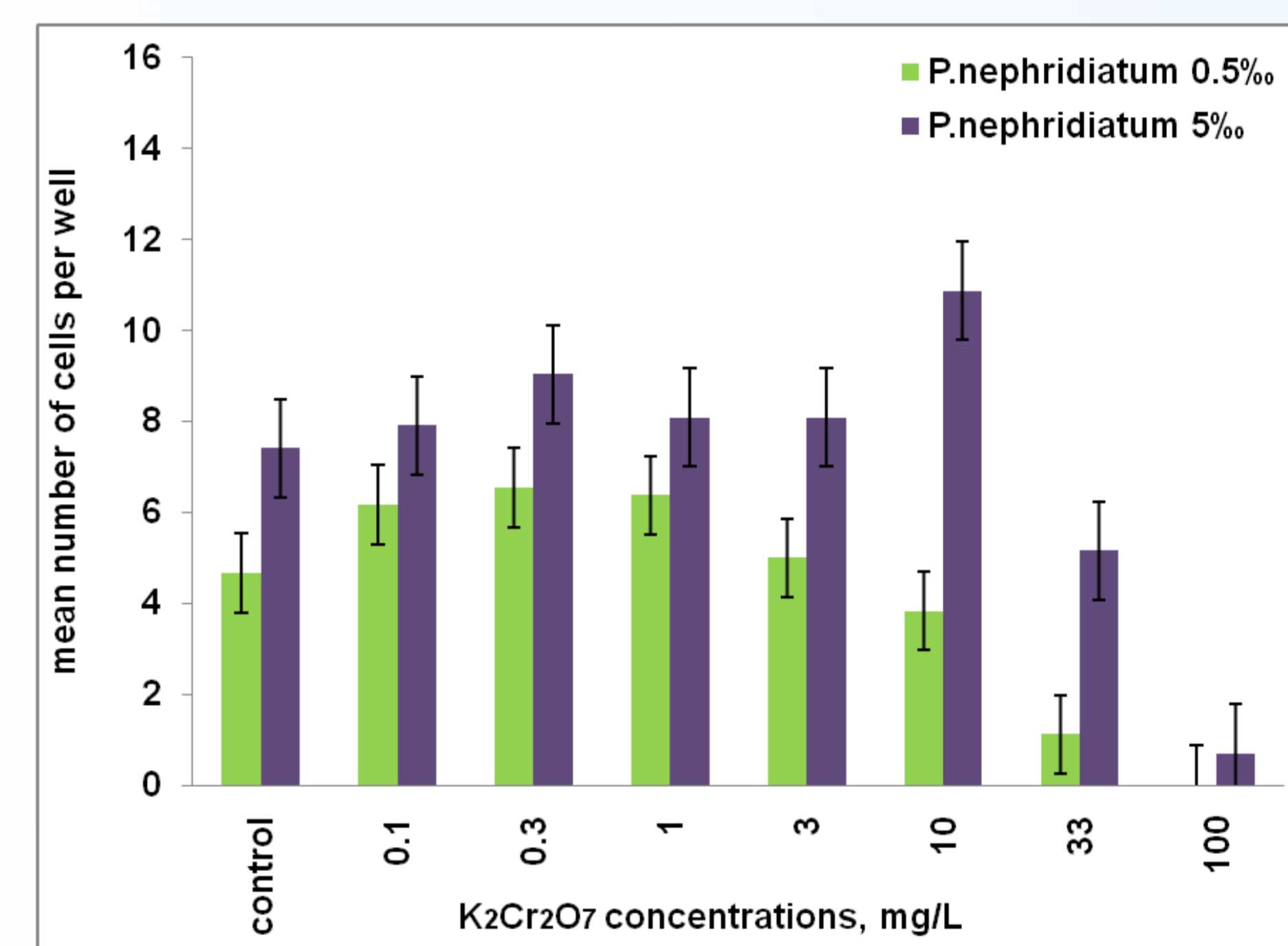
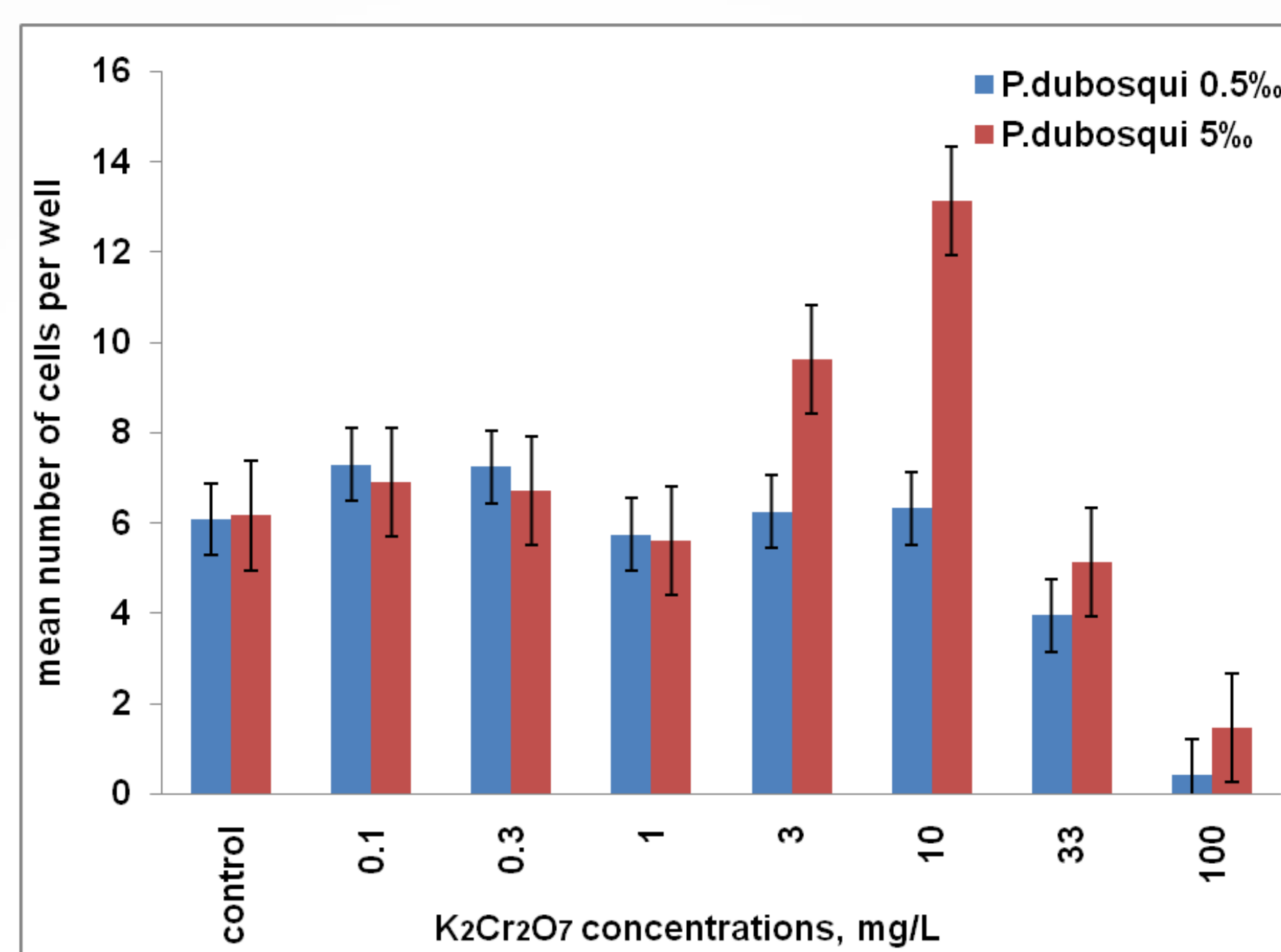


Fig.1. Effects of potassium dichromate on number of cells of two *Paramecium* species (*P. dubosqui* and *P. nephridiatum*, both acclimated to salinities 0.5 and 5‰) after 72h exposures; N = 24 (4 independent experiments, each performed in 6 replicates), bars represent mean ± standard deviation.

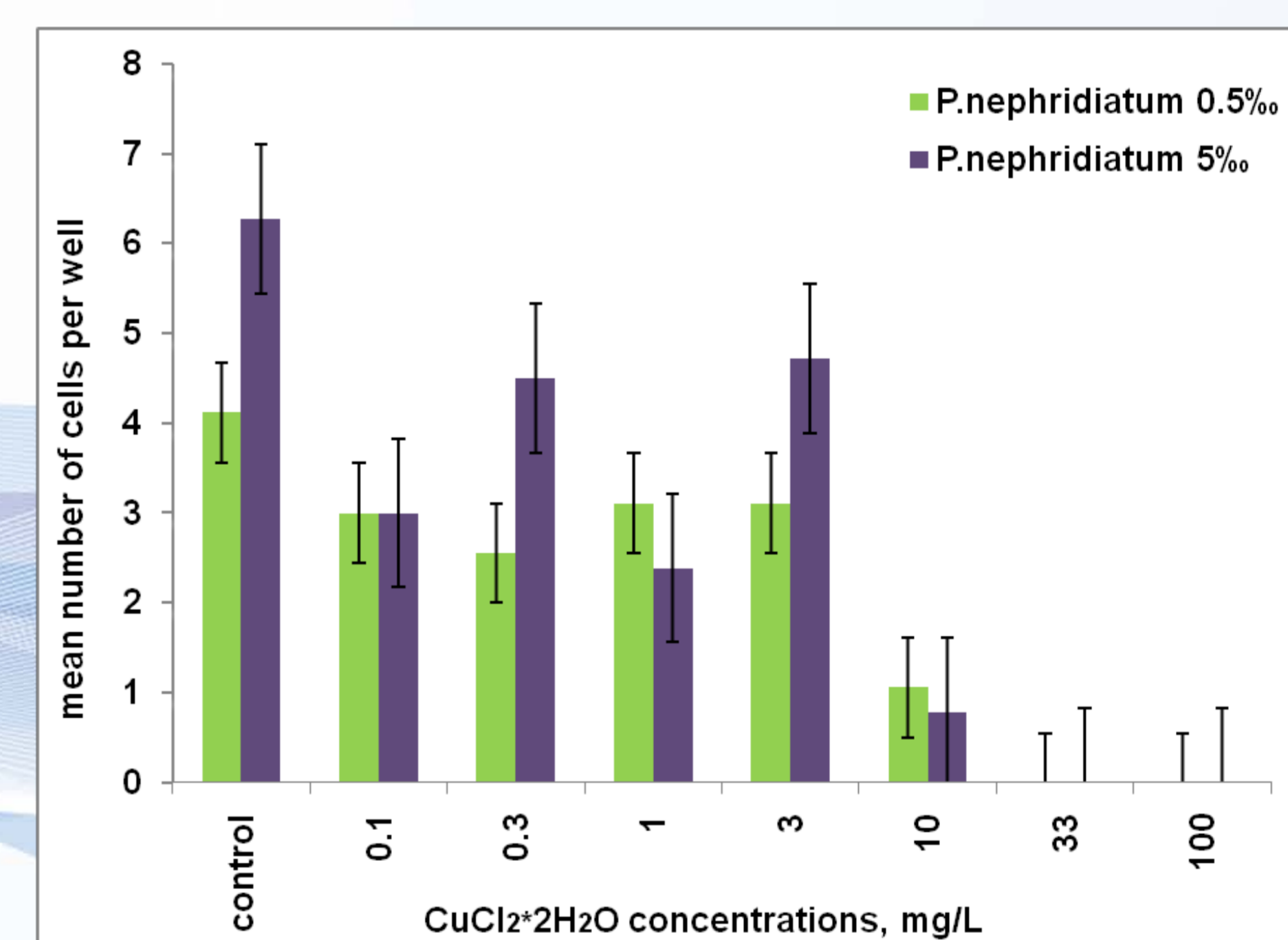
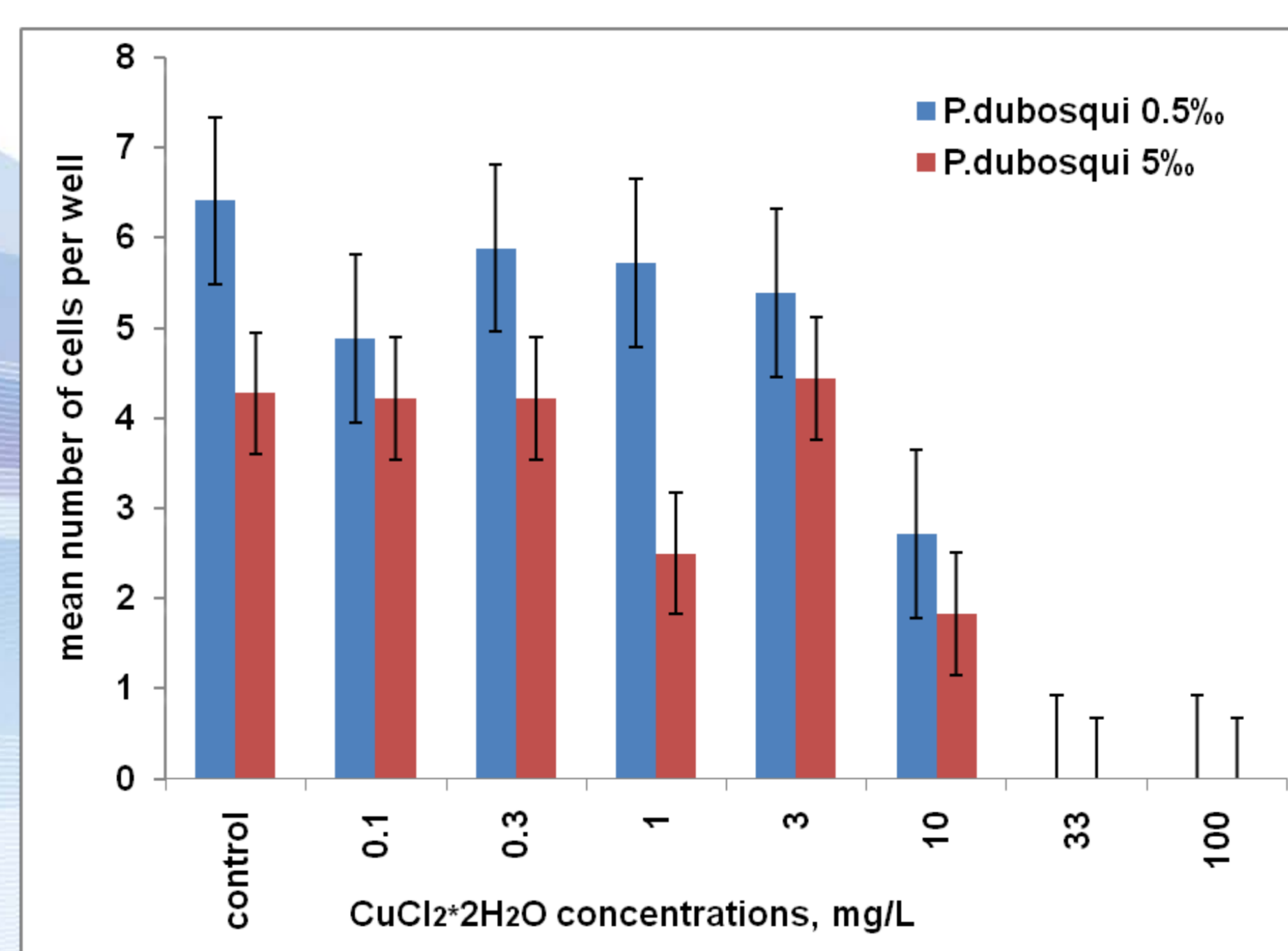


Fig.2. Effects of copper dichloride dihydrate on number of cells of two *Paramecium* species (*P. dubosqui* and *P. nephridiatum*, both acclimated to salinities 0.5 and 5‰) after 72h exposures; N = 18 (3 independent experiments, each performed in 6 replicates), bars represent mean ± standard deviation.

## CONCLUSIONS

- The data obtained shown that both cultures of euryhaline ciliates can be used for assessment the combined effects of salinity and metal toxicity, providing quite stable results.
- Interferences between the salinity and toxic effects were recorded (e.g. systematically observed „stimulations“ - hormesis-like effect in tests with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> at higher salinity at both species; variable “species-specific” responses in the experiments with CuCl<sub>2</sub>)
- The proposed approach can be used for further studies of other toxicants and the interactive effects of increased salinities