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Effect of zinc on the heterotrophic  
bacteria in sea water

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Utjecaj cinka na populaciju heterofinih bakterija

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INTRODUCTION

The development of analytical methods (Florence and Batley, 1976; Nürnberg, 1977) rendered possible a control of disturbances caused by different pollutants of anthropogenic origin particularly heavy metals (Zn, Cd, Pb and Cu) in biogeochemical cycle.

Copper and zinc, as toxicants, occupy a special position among the heavy metal group (Sunda and Guillard, 1976; Stoecker *et al.*, 1986; Stauber and Florence, 1987). However, at the same time they are essential elements (Anderson *et al.*; 1978) as far as different marine organisms are concerned.

Gillespie and Vaccaro (1978) studied bacterial activity as affected by copper and organic complexing agents and established the non-linearity of the activity decrease with the increase of copper concentrations. Kim (1985) found lower toxicity of cadmium and copper to bacteria in surface sea water film in relation to subsurface sample. This was associated with the enrichment of complexing agents in the surface microfilm.

The aim of this paper is to study the effects of zinc on bacteria since the data on the effects of this toxicant are scarce.

MATERIAL AND METHODS

Sea water for the experiment was taken from the area far off the municipal and industrial effluents. All polyethylene and glass ware, coming in contact with the sample was prepared by the procedure given by Mart (1982).

Zinc concentrations in the sample were determined by differential pulse anodic stripping voltammetry using a PAR-174A Polarographic Analyzer in combination with a 303 SMD-electrode under the following conditions:

- Hanging mercury drop mode
- Accumulation potential: 1.25 V vs Ag/AgCl
- Accumulation time: 240 s

The ionic zinc concentrations ( $Zn^{2+} = 0.42 \mu\text{g dm}^{-3}$ ) were determined in the untreated sample at pH = 8.27 while for the total zinc concentration ( $Zn^{2+} = 0.73 \mu\text{g dm}^{-3}$ ) the sample was acidified to pH = 4.6.

Sample was divided into eight earlier prepared quartz glass flasks. A flask with the sample and no zinc added was used as control of bacterial growth. Zinc in the form of  $ZnCl_2$  (Table 1) was added to the remaining seven flasks.

Table 1. Added Zn concentrations

Flask	Zn concentrations ( $\mu\text{g dm}^{-3}$ )
1	*
2	0.024
3	0.24
4	0.48
5	0.72
6	2.40
7	24.0
8	240

\* = control (natural concentrations of zinc)

The experiment lasted for five days, at 20°C temperature in the dark. The number of bacteria was observed by inoculation on the ZoBell's 2216 medium (ZoBell, 1946). The experiment was triplicated.

Bacterial growth rate was determined from the experimental curve ( $N_t = N_0 e^{-kt}$ ), where all symbols have conventional meanings.

Difference in bacterial growth rates were statistically tested by the analysis of variance (ANOVA) and SNK-test.

## RESULTS AND DISCUSSION

Added zinc concentrations of 0.024 to 0.72  $\mu\text{g dm}^{-3}$  caused no significant drop of growth rate coefficient (k) of bacterial population. Further added concentration of 2.4 to 0.72  $\mu\text{g dm}^{-3}$  resulted in a sudden decrease of growth rate coefficient. Next significant drop in growth coefficient rate was recorded at added zinc concentration of 24 to 240  $\mu\text{g zinc per dm}^{-3}$  (Fig. 1).

These results were confirmed by a statistical data analysis (Fig. 2) which pointed to the statistically significant difference in growth rate coefficients between I, II and III group. However, the intragroup differences were not significant.

The phenomenon of growth coefficient not being significantly changed by adding up to  $0.72 \mu\text{g dm}^{-3}$  zinc concentrations may be associated with the complexation of added zinc with organic ligands present in the sea water sample.

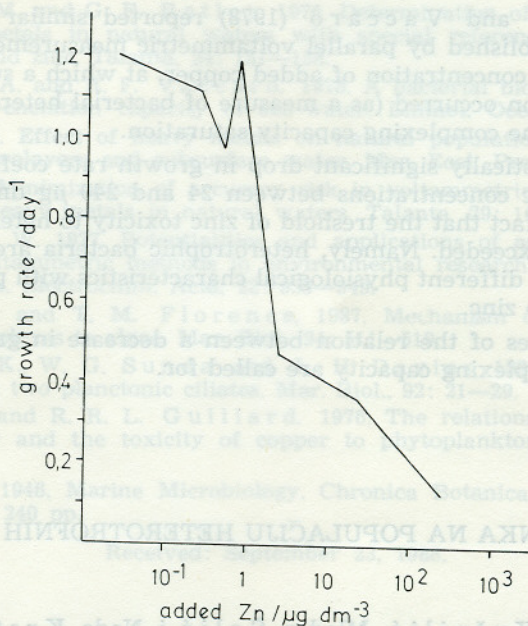


Fig. 1. Dependence of the coefficient of bacterial growth rate on added zinc concentrations.

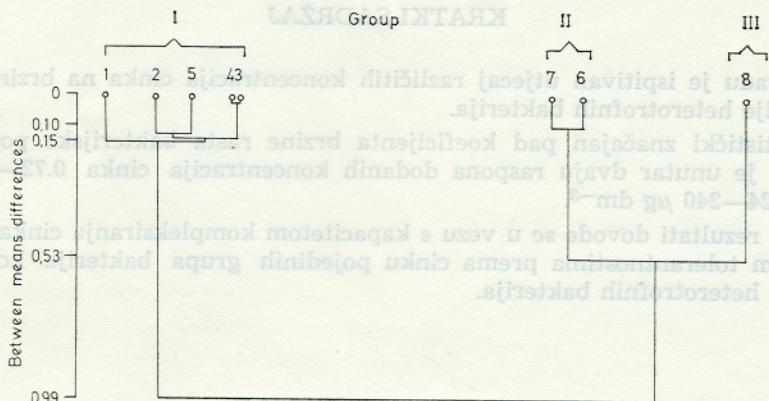


Fig. 2. Dendrogram of arithmetical means of growth rate coefficients. Differences of arithmetical means between I, II and III groups are significant (ANOVA and SNK-test) Figs. 1 through 8 present added zinc concentrations according to Table 1 where bacterial growth was observed.

The effect of complexation is a buffer effect of the sea water on zinc, that is the concentration of added zinc cannot increase and does not affect bacteria. It is very likely that toxicity of ionic zinc becomes pronounced only after saturation of complexing capacity. This may account for the sudden drop in growth rate coefficient.

Gillespie and Vaccaro (1978) reported similar phenomenon for copper. They established by parallel voltammetric measurement of complexing capacity, that the concentration of added copper, at which a sudden decrease of glucose degradation occurred (as a measure of bacterial heterotrophic activity), corresponded to the complexing capacity saturation.

Another statistically significant drop in growth rate coefficient was recorded at added zinc concentrations between 24 and 240  $\mu\text{g dm}^{-3}$ . This may be explained by the fact that the threshold of zinc toxicity to heterotrophic bacteria was once again exceeded. Namely, heterotrophic bacteria are a heterogeneous bacterial group of different physiological characteristics with probably different tolerance levels to zinc.

Further studies of the relation between a decrease in growth rate coefficient and the complexing capacity are called for.

## UTJECAJ CINKA NA POPULAČIJU HETEROTROFNIH BAKTERIJA

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### KRATKI SADRŽAJ

U radu je ispitivan utjecaj različitih koncentracija cinka na brzinu rasta populacije heterotrofnih bakterija.

Statistički značajan pad koeficijenta brzine rasta bakterijske populacije utvrđen je unutar dvaju raspona dodanih koncentracija cinka 0.72—2.40  $\mu\text{g dm}^{-3}$  i 24—240  $\mu\text{g dm}^{-3}$ .

Ovi rezultati dovode se u vezu s kapacitetom kompleksiranja cinka kao i s različitim tolerantnostima prema cinku pojedinih grupa bakterija koje čine skupinu heterotrofnih bakterija.

## REFERENCES

- Anderson, M. A., N. M. F. Morel and R. R. L. Guillard. 1978. Growth limitations of a coastal diatom by low zinc ion activity. *Nature*, 276: 70—71.
- Florence, T. M. and G. E. Batley. 1976. Determination of the chemical forms of trace metals in natural waters with special references to copper, lead, cadmium and zinc. *Talanta*, 24: 151—158.
- Gillespie, P. A. and R. F. Vaccaro. 1978. A bacterial bioassay for measuring the copper-chelation capacity of sea-water. *Limnol. Oceanogr.*, 23: 543—548.
- Kim, S. J. 1985. Effect of heavy metals on natural populations of bacteria from surface microlayers and subsurface water. *Mar. Ecol. Prog. ser.*, 26: 203—206.
- Mart, L. 1982. Minimization of accuracy risk in voltammetric ultratrace determination of heavy metals in natural waters. *Talanta*, 29: 1035—1040.
- Nürnberg, H. W. 1977. Potentialities and applications of advanced polarographic and voltammetric methods in environmental research and surveillance of toxic metals. *Electrochim. Acta*, 22: 935—949.
- Stauber, J. L. and T. M. Florence, 1987. Mechanism of ionic copper and copper complexes to algae. *Mar. Biol.*, 94: 511—519.
- Stoecker, D. K., W. G. Sunda and L. H. Davies. 1986. Effects of copper and zinc on two planctonic ciliates. *Mar. Biol.*, 92: 21—29.
- Sunda, W. G. and R. R. L. Guillard. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. mar. Res.*, 34: 511—529.
- ZoBell, C. E. 1946. *Marine Microbiology*. Chronica Botanica Company of Waltham Mass., 240 pp.

Received: September 23, 1988.

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#### MATERIAL AND METHODS

BILJEŠKE-NOTES, izdaje Institut za oceanografiju i ribarstvo, Split; izlazi povremeno. Odgovorni urednik: Dr Mira Zore-Armanda. Naklada 600 primjeraka. Tisak  
»Slobodna Dalmacija«, Split