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Effect of Copper and Cadmium on Natural Populations of Bacteria from Surface Microlayers

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중금속이 해양의 표층세균군집에 미치는 영향에 관하여

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ABSTRACT

The effect of the heavy metals copper and cadmium on the natural populations of surface microlayer and subsurface water was investigated. Two microbiological parameters, number of colony-forming bacteria and ^{14}C-glucose uptake rate, were evaluated. The two natural bacterial populations showed different tolerances of the heavy metals. The inhibition of bacterial growth and activity occurred more strongly in the 1 m-depth samples than in neuston populations. The results support the existence of autochthonous bacterioneuston populations in marine environment.

INTRODUCTION

Although a large number of metals are essential for growth, they can also effect considerably harmful on living cells. It is mainly due to that heavy metals build complexes with protein molecules and make them inactive, for example, inactivation of enzymes. Many heavy metals are detrimental to microorganisms in low concentractions, which are present in natural waters (Mills and Colwell, 1977). There are also reports on the adaptation of microorganisms to heavy metals (Azam *et al.*, 1977). This phenomenon has a important meaning for the microbial ecology in polluted ecosystems.

Accumulations of bacteria in the surface microlayer have been reviewed by many authors (Sieburth, 1979; Norkrans, 1980). Enrichments of toxic substances, such as heavy metals, in surface microlayers have been also described from various aquatic ecosystems (Baker and Zeitlin, 1972; Lion and Leckie, 1982). This means that the physiological state of neuston can be considerably stressed by toxic substances. For the better understanding of the biological function of neuston in the marine environment, the influence of high concentrations of heavy metals on the natural populations of bacterioneuston was investigated.

MATERIAL AND METHODS

Seawater was obtained only in calm days from the innermost Kiel Fjord (Baltic Sea, FR of Germany). Surface microlayer samples were collected with a sterile circular screen (0.1256 m^2, 16 mesh, wire diameter 0.35 mm, stainless steel). The thickness of the sampled surface microlayer was 430 μm. Sampling

from a depth of 1 m was conducted with a sterile 5-liter glass bottle. The two water samples were filtered through a stainless steel sieve (mesh size 75 μm) before being used to remove larger zooplankton and detritus.

The influence of Cd- and Cu-salts was determined on the basis of bacterial survival (colony-forming-units) and glucose uptake rate. Sterile standard solutions of heavy metals, Cd $(NO_3)_2 \cdot 4H_2O$, were made and individually mixed with either 500 ml neuston sample or with the subsurface sample. Two different Cd-concentrations and a control were tested for each sample. They were 1 and 10 ppb (w/v) (10 ppb Cd=10 μgCd. l^{-1}=27.444 μg Cd $(NO_3) \cdot 4H_2O$. l^{-1}). Two concentrations were similarly tested for Cu and reflected 10 and 100 ppb (w/v) (100 ppb Cu=100 μg Cu. l^{-1}=118 μg $CuCl_2 \cdot 2H_2O$ l^{-1}). These concentrations are about 10 and 100 times greater than the natural concentrations of 2 m depth in the investigative area (Kremling *et al.*, 1979). The samples were incubated for 24 hrs at room temperature and continually shaken. Immediately previous to the addition of heavy metals the colony-forming-units and ^{14}C-glucose uptake rate of the bacteria were determined.

Colony-forming-units on ZoBell agar plates with 17‰ salinity were counted after incubation of 14 days. For the measure of the glucose uptake rate (0.2 ml D-(u-^{14}C)-glucose solution (=2.5 μgC) to 3 parallels of samples from each glass were added and incubated 1 hour at room temperature. One of the 3 parallels was set up as a control (fixed with 0.05 ml formalin at the start of the incubation) in order to determine the amount of radioactive substances adsorbed. After incubation the samples were fixed with formalin and then filtered through membrane filters (pore size 0.2 μm, Satorius) and rinsed twice with isotonic solution. The filters were then put into scientillation vials with 10 ml of Dioxan-cocktail and counted in a liquid scintillation counter (Betascint 5000, Berthold and Frieseke) until the statistical counting error was $\pm 1\%$.

RESULTS AND DISCUSSION

Fig. 1 and 2 present the influence of two different concentrations of copper on the

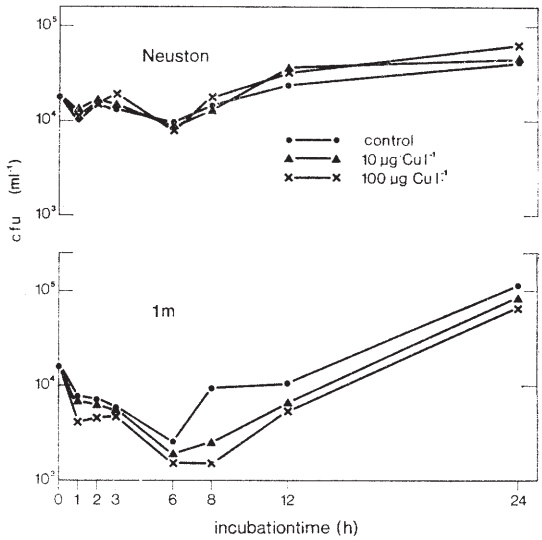

Fig. 1. Effect of copper on the number of colony-forming bacteria.

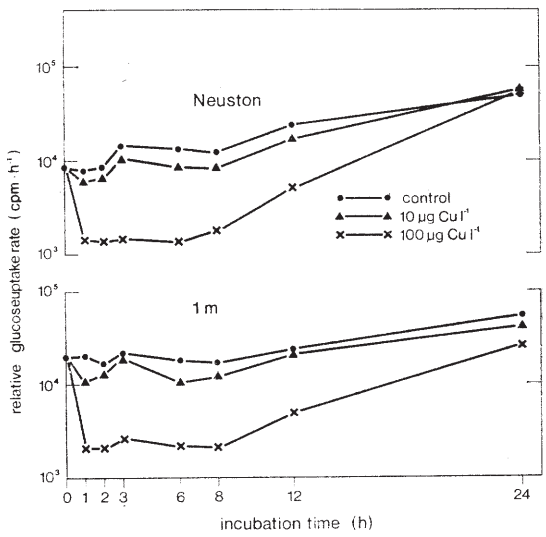

Fig. 2. Effect of copper on the glucose uptake rate of bacteria.

bacterial incorporation of radioactively labelled glucose as well as on the number of colony-forming-units. The stronger inhibitory effect of copper was reflected on the bacterial uptake rate of glucose. After 24h of incubation the inhibiting effect on the sample from a depth of 1 m was still apparent, whereas the neuston sample took up glucose in the copper-supplemented water to the same degree as did the control. In regard to the colony-forming-units in the neuston sample no significant differences were observed between the control and the copper-containing samples. In contrast, for bacteria from the subsurface water there were correspondingly less colony-forming-units counted with an increase in the quantity of supplemented copper.

The effects of cadmium on bacteria are exhibited in Fig. 3 and 4. At the lower Cd-concentration bacterial growth was somewhat inhibited up to an incubation time of 6 h, whereafter no inhibition was observed. At the higher cadmium concentration the inhibiting effect in the neuston sample increased with increasing incubation time. However, the number of colony-forming-units remained within the same order of magnitude as the control. In contrast, a considerable difference between the control and the Cd-supplemented sample was noted after 24 h incubation for the subsurface water, whereby the number of colony-forming-units is up to two orders of magnitude smaller than in the control. With respect to glucose incorporation no remarkable inhibition was found in neuston, but distinct differences could be observed after 24 h between the cadmium-supplemented subsurface water and the control.

Seasonal investigations of different microbiological parametes revealed as a rule higher numbers of bacteria in surface microlayers than in subsurface waters (Kim, S.J., Ph.D. Thesis, Kiel University, FRG, 1983). Chemical components including pollutants such as heavy metals are likewise enriched in the surface microlayer (Waldichuk, 1982). The enrichment of such toxic substances can exert a tremendous influence on the organisms living in this extreme biotope.

Two different phenomena were observed in

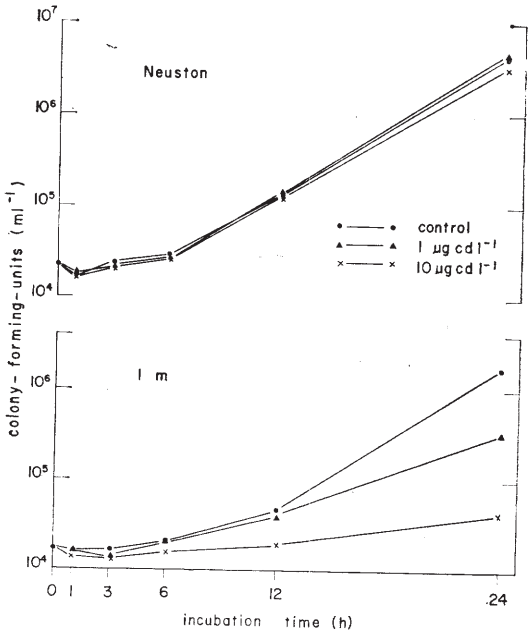

Fig. 3. Effect of cadmium on the number of colony-forming bacteria.

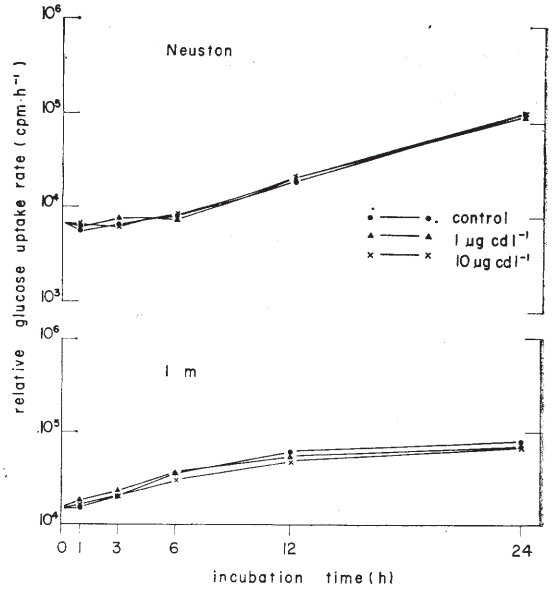

Fig. 4. Effect of cadmium on the glucose uptake rate of bacteria.

this investigation regarding the heavy metal influence on natural bacterial populations. With the addition of cadmium the number of living bacteria and the glucose uptake rate decreased with time. Furthermore, the inhibition of growth and activity occurred more strongly in the 1 m samples than in neuston. A similar effect was observed with the supplement of copper. Accordingly, neuston samples distinctly demonstrate a lesser inhibition with respect to copper and cadmium than do the water samples from 1 m. There are several possible interpretations for this observation. The concentration-dependent toxicity of a heavy metal can be reduced through various means: through complex-formation with organic compounds (Milanovich and Wilson, 1975; Benes *et al.*, 1976), through bacterial incorporation and accumulation (Doyle *et al.*, 1975) and through biological metal-transformation (Brunker and Bott, 1974; Holm and Cox, 1975) In this specialized biotope the toxic effects of heavy metals could possibly be reduced or masked through the formation of complexes of the metals with organic substances which are enriched in the surface microlayer.

On the other hand, an adaptation (or selection) of bacteria to heavy metal occurs in the marine environment (Azam et al., 1977). Bacteria originating from metal contanining waters or sediments demonstrate a stronger tolerance to heavy metals than do those from uncontaminated environments (Mills and Colwell, 1977). Thorman and weyland (1979) thus obtained different sensitivities for bacteria in an estuary and in the open ocean in regard to the heavy metals cadmium and lead. Because heavy metals are probably continuously present, bacterial adaptations to these substances can be expected, as has been observed in this investigation. It is also considerable that the high heavy metal enrichments in surface microlayers which are from 10 to 100 times higher than in the underlying water (Waldichuk, 1982), can be incorporated or absorbed to a certain degree by bacteria and eventually introduced to the food chain.

The existence of a surface microlayer biotope is only definable under the assumption that it distinguishes itself in character and composition from the underlying water. From this viewpoint the different tolerances between the two investigated water samples to the heavy metals support the existence of autochthonous bacterioneuston populations in the marine environment.

적 요

중금속(구리와 카드뮴)이 해양의 표층수와 1 m 깊이에서 채취된 미생물군집에 미치는 영향에 관하여 연구되었다. colony 형성균수와 ^{14}C-glucose uptake rate가 배양시간에 따라 각각 측정되었다. 해양에서 채취된 두개의 미생물군집은 서로 상이한 생리적 반응을 나타내었다. 이 결과로서 해양생태계에 토착적인 bacterioneuston population의 존재가 가능함을 추정할 수 있다.

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