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Growth of four planktonic algae from brackish water in the presence of heavy metals

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Abstract

The influence of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn on the algal growth was studied. *Chlorella vulgaris, Oocystis submarina, Stichococcus bacillaris* and *Cyclotella meneghiniana* were exposed to the concentrations of metals of 0–10.0 mg dm$^{-3}$. Three groups of metals were distinguished. The first group constitute the most toxic metals, cadmium and copper, which did not show promoting effect on algal growth; NOEC and EC$_{10}$ values were lower than 0.1 mg dm$^{-3}$; EC$_{50}$ was always lower than 1 mg dm$^{-3}$. The second group consists of zinc, cobalt, nickel and lead, which showed an intermediate effect on algal growth; stimulation at low concentrations and total inhibition at high ones, usually above 5 mg dm$^{-3}$. The least toxic group consists of manganese and iron; they never caused a 50 % growth reduction up to 10 mg dm$^{-3}$.

**Key words:** heavy metals, toxicity, growth, planktonic algae, green algae, diatom

1. Introduction

Many heavy metals are necessary for proper course of various life processes in plant cells. Being the components of many enzymes and proteins they take part in specific metabolic pathways. Moreover, they affect synthesis of the growth hormones or membrane permeability at cellular level (Zurzycki, Michniewicz 1985; Kabata-Pendias, Pendias 1993). This group of metals consists mainly of chrome, tin, zinc, cobalt, manganese, copper, molybdenum, nickel, vanadium and iron. The other group constitute metals of unknown physiological function, such as cadmium, lead, mercury, silver and gold (Furnes, Rainbow 1990). Usually the metals are necessary at relatively low concentration and their higher level in the cell environment could become toxic. The influence of heavy metals on phytoplankton organisms depends chiefly on their concentration and chemical form (Sunda 1990). It was found that the toxicity of heavy metals to many algal species is a function of free ion concentration (Sunda, Guillard 1976; Brand et al. 1986; Morgan, Stumm 1991). The chemical speciation of the metals depends on many factors like organic and inorganic ligands. Among them an important complexing factor present mainly in sea water are chloride ions (Sunda et al. 1978; Bruland 1983; Bruland, Franks 1983; Latala, Surosz 1998). Therefore, it is especially interesting to recognize the sensitivity of brackish Baltic algae to increased concentrations of heavy metals. Algae adsorb efficiently metals to surface ligands on cell walls (Knauer et al. 1997), could enzymatically produce phytocelatin, polypeptides like metallothionein in animal, which chelate metals (Ahner et al. 1995) and a number of species are known to exude metal binding ligands...
(Gledhill et al. 1997). These are likely to have an effect on the availability of metals and cause the differences in algal response to heavy metals.

Large quantities of pollutants of different toxicity, among them heavy metals from industrial and domestic sewage, are released annually into coastal marine waters. Once introduced to the marine environment, the metals are not withdrawn and could be only temporarily inactivated as a result of the formation of various inorganic or organic connections (Kowalewska 1988, 1994; Li 1991). In the coastal waters, always subjected to contamination inflow, the increased concentrations of heavy metals could affect algal cells for a longer period. To determine the toxicity of different compounds so called acute tests are usually applied. A tested organism is subjected to the short-term influence of a given compound, without previous adaptation (ISO 1993, 1995). In our investigations we applied a 7 days’ acclimation period prior to a long-term test. As opposed to acute tests, in long-term tests algae tolerance to heavy metals was investigated and such tests could better reflect the response of algae to permanent presence of heavy metals in the environment. Reports indicate that some algae are capable of developing resistance to toxic chemicals after prolonged exposure (Fisher 1981).

The aim of the present experiments was to estimate the effect of 8 heavy metals on growth and chlorophyll a content in four phytoplanktonic Baltic algae cultivated in the medium without chelators.

2. Materials and methods

The investigations were performed on four algal species derived from natural phytoplankton assemblages of the Gdańsk Bay. Three green algae, Chlorella vulgaris Beijerinck (LA–1/685), Oocystis submarina Lagerheim (LA–2/682), Stichococcus bacillaris Nägeli (LA–9/682) and one diatom, Cyclotella meneghiniana Kützing (LA–10/989), were isolated from surface water (Latała 1993). The back-up stock cultures of each strain were set up several months prior to the experiments and maintained as unicellular in collection of algal cultures of Marine Plant Ecology Laboratory, University of Gdańsk. The algae were grown in complete f/2 medium (Guillard 1975) at 18±1°C with photosynthetically active radiation (PAR) of 30 µmol photons m⁻²s⁻¹ and photoperiod 16:8 L:D. No tests were conducted to assess whether cultures were axenic, though bacterial contamination was not apparent under the light microscope. Because the cultures were handled aseptically throughout, it is presumed that those bacteria, which may have been present, were associated with the algal cells at the time of isolation.

The investigations were performed on batch cultures of algae. To prepare the final medium seawater with salinity of 7.6 psu was used. Seawater was taken from the surface water of the Gdańsk Bay at the station GN (54°32’N and 18°48’E) situated in the central part of the bay and was filtered by glass-fibre (Whatman GF/C). The flasks used for the culture were initially cleaned with detergent and soaked in 1 N hydrochloric acid for at least 48 h. Then they were rinsed with glass distilled water and sterilized at 120°C for about 2 h. The media were sterilized by standard autoclaving procedure (20 min, 120°C, 1.2 Atm) and were poured into the sterile flasks in a cabinet with aseptic blow of air and then inoculated. Stock solutions of macronutrients and trace elements were made from reagent grade chemicals and Pyrex glass distilled water and thousandfold more concentrated than in the final medium. To obtain f/2 medium 1 cm³ of each stock solution was added to 1 dm³ of seawater. The concentrations of heavy metal stock solutions of ZnCl₂, CoCl₂ 6H₂O, MnCl₂ 4H₂O, CuCl₂ 2H₂O, NiCl₂ 6H₂O, FeCl₂ 6H₂O, CdCl₂ and PbCl₂ were 100 times higher than test concentrations and prepared aslo from analytical grade chemicals and Pyrex glass distilled water.

Stock and experimental media were prepared as f/2 but without EDTA and with addition of a heavy metal. EDTA was omitted because as a chelating factor it forms complex compounds with metals, also with the heavy ones, and significantly modifies their influence on the organisms (Anderson, Morel 1982). Trace elements were present at the following concentration per dm³ of the medium: Fe 0.65 mg; Cu 2.5 µg; Zn 5 µg; Co 2.5 µg; Mn 50 µg; Mo 2.5 µg. Heavy
metals: Zn, Co, Mn, Cu, Ni, Fe, Cd and Pb were added in the following concentrations: 0.1, 0.5, 1.0, 5.0 and 10.0 mg dm$^{-3}$. In control cultures the metal tested was either absent or its concentration resulted from the medium composition (trace element). In the latter case the metal concentration, with the exception of Fe, was many times lower than the lowest concentration tested. In each situation the metal concentration in the control cultures was arbitrary accepted as 0.

To determine algal tolerance to the presence of heavy metals long-term tests were carried out using stock and experimental cultures. Stock cultures were exposed to the presence of heavy metals for 1 week. The inocula for experimental cultures were taken from the stock cultures and added to the flasks to give initial cell density of $10^4$ cells cm$^{-3}$ (C. vulgaris and S. bacillaris) or $3 \times 10^3$ cells cm$^{-3}$ (O. submarina and C. reinharniana). The experimental cultures were incubated for 9 days in 50 cm$^{-3}$ glass Erlenmeyer flasks stopped with cotton plugs and containing 30 cm$^{-3}$ of medium. All test concentrations and controls were run in triplicate. Stock and experimental algal cultures were incubated in a growth chamber at 24±1°C and received 120 μmol photons m$^{-2}$s$^{-1}$ of PAR (measured using a quantum-meter LiCor 165 with a cosine collector) for 16 h d$^{-1}$ from 250 W high-pressure sodium lamps. The cultivation vessels were manually and gently shaken once a day.

In experimental cultures growth response was measured as the cell number on 6th and 9th day, i.e. at exponential growth phase. Samples of 1–2 drops were removed from each culture at sterile conditions and cell number was determined microscopically in a Bürker or Fuchs-Rosenthal hemocytometer counting chamber. Since the counts were in good agreement, the data were pooled. Replication was such that coefficients of variations within treatments were generally less than 10%. On 9th day chlorophyll a level in culture was determined spectrophotometrically according to Strickland, Parsons (1972). A known volume of algal culture was filtered by Whatman GF/C filters. Until the time of measurement the filters were stored in darkness at −10°C. Absorbance of the extracts were determined on spectrophotometer VSU 2–G at wavelengths of 630, 647, 664 and 750 nm. Chlorophyll a concentrations were calculated from the equations given by Jeffrey, Humphrey (1975). In addition, for exponential growth phase the relative growth rate ($K_2$) for each test culture was calculated (Guillard 1973) from the equation:

$$K_2 = \ln \left( \frac{N_1}{N_0} \right) \left( \frac{t_1 - t_0}{t_0} \right)$$

Where: $K_2$ – growth rate; $N_0$ – initial cell density (6th day); $N_1$ – final cell density (9th day); $t_1$ – $t_0$ – time (in days) between succeeding cell number measurements.

To compare the toxic effects of heavy metals on the algae tested, the following values were calculated: no observed effect concentration NOEC (the highest concentration tested at which there is no statistically significant reduction of growth relative to the controls), effective concentration EC$_{10}$ or EC$_{50}$ (the concentration of the metals which results in a 10 or 50% reduction in population growth relative to the controls) and totally growth inhibition TGI (growth reduction below 5% in relation to the control). The values of EC$_{10}$ and EC$_{50}$ were calculated from the cell number after 9 days of cultivation.

3. Results

During the experiments the density of cell populations were measured on 6th and 9th days of cultivation and on the last day chlorophyll a content was determined. From the results obtained relative growth rates ($K_2$) were calculated. It appeared that both the measured and calculated parameters were equally relevant in illustrating the effect of the metal influence in different experimental systems, e.g. Figs 1–3. Since it was assumed that population density is the best criterion of the metal influence on the growth of algal
cultures, the results of the investigations were presented only as the cell number on the ninth day of cultures. This parameter is often used in literature because the changes in cell number are the most direct reflection of algal response to toxic influence of metals. To facilitate the comparisons of the effects of metals on phytoplanktonic cultures the data were presented as percentage of the control cultures.

**Cadmium**

Algal cultures responded to cadmium with a very strong decrease in growth with an increase in metal concentration. In *C. vulgaris* (Fig. 4A) and *O. submarina* (Fig. 4B) 35 % growth reduction in relation to the control was observed at 0.1 mg dm$^{-3}$. Higher concentrations completely inhibited the cell growth. In *C. meneghiniana* a similar reaction was found. The lowest Cd concentration (0.1 mg dm$^{-3}$) caused a decrease in cell number to 33 % as compared to the control (Fig. 4D). At higher concentrations the culture growth was almost totally stopped. In *S. bacillaris* a gradual decrease in cell number with an increase in Cd concentration was noted (Fig. 4C), e.g. at 0.1 and 10 mg dm$^{-3}$ a 60 and 95% growth reduction in relation to the control.
Fig. 3. *C. meneghiniana* cell number (D9) and relative growth rates (K2) after 9 days of culture at different Cu concentration.

Fig. 4. Cell number in *C. vulgaris* (A), *O. submarina* (B), *S. bacillaris* (C) and *C. meneghiniana* (D) after 9 days of culture at different Cd concentration.
**Copper**

*O. submarina* was the most sensitive species to copper influence: at 0.5 mg dm\(^{-3}\) the growth of this alga was totally stopped (Fig. 5B). *C. meneghiniana* (Fig. 5D) showed a somewhat greater resistance to copper. The growth was almost completely stopped at 1 mg dm\(^{-3}\). The least sensitive were the strains of *C. vulgaris* (Fig. 5A) and *S. bacillaris* (Fig. 5C); an increase in Cu concentration from 0.1 to 1 mg dm\(^{-3}\) caused a gradual decrease in population densities. At 1 mg dm\(^{-3}\) a 60 and 70% reduction in growth of *C. vulgaris* and *S. bacillaris* was noted, respectively. Higher Cu concentrations completely inhibited the growth of the both species.

**Zinc**

Zinc affected algal species in a different way. In *C. vulgaris* an increase in zinc concentration caused a strong growth inhibition (Fig. 6A). Concentrations of 0.1 and 0.5 mg dm\(^{-3}\) did not affect cell number, but at 1 mg dm\(^{-3}\) a 70% reduction of growth was observed and at higher concentrations, 5 and 10 mg dm\(^{-3}\), the algal growth was completely stopped. In *O. submarina* (Fig. 6B) an increase in zinc concentration in the range of 0.1–1 mg dm\(^{-3}\) caused algal
growth stimulation of about 35% as compared to the controls. However, the further increase in zinc concentration, 5 and 10 mg dm$^{-3}$, resulted in a dramatic inhibition in population density. In *S. bacillaris* (Fig. 6C) all the concentrations tested stimulated strongly the growth of cultures. At 1 and 5 mg dm$^{-3}$ the cell densities were higher than in the control culture by a factor of ca. 2.5. At the remaining concentrations the growth promoting effect was lower but also evident. In *C. meneghiniana* (Fig. 6D) the results of the experiments were similar to those of *O. submarina*. At low zinc concentration an increase in culture density in relation to the control was observed. The highest stimulation, above twice as large, was noted at 0.1 mg dm$^{-3}$. The highest concentrations, 5 and 10 mg dm$^{-3}$, almost completely inhibited the growth of the algae tested.

**Cobalt**

In *C. vulgaris* there were no significant differences in population density at 0.1 and 0.5 mg dm$^{-3}$ of Co (Fig. 7A) as compared to the control cultures. Higher concentrations strongly reduced population growth. At 10 mg dm$^{-3}$ the growth was almost completely stopped. Similar results were noted in *O. submarina* (Fig. 7B), but a marked inhibition of population growth was
observed only when the culture was treated with 5 mg dm$^{-3}$ of Co. In *S. bacillaris* (Fig. 7C) an increase in Co concentration from 0.1 to 1 mg dm$^{-3}$ modified the cell number in a very small degree. Higher concentrations (5 and 10 mg dm$^{-3}$) caused a gradual reduction in algal growth, 40 and 65 % respectively, but their effects were considerably weaker than in the other species tested. In *C. meneghiniana* (Fig. 7D) Co in the range between 0.1 and 1 mg dm$^{-3}$ slightly stimulated the algal growth. The maximum effect, 40 % in relation to the control, was observed at 0.5 mg dm$^{-3}$. Higher Co concentrations, 5 and 10 mg dm$^{-3}$, caused a marked, 75 and 90% reduction in population densities, respectively.

**Nickel**

In green algae cultures a similar reaction to nickel was observed. *C. vulgaris* (Fig. 8A) showed to be the most sensitive strain. The cell number dropped by above a half in relation to the control already at 0.1 mg dm$^{-3}$. Higher concentrations stronger and stronger decreased the culture density and at 5 and 10 mg dm$^{-3}$ the growth was almost totally inhibited. Similar influence was
observed in *O. submarina* (Fig. 8B) but the degree of growth inhibition was markedly lower. At 0.1 and 1 mg dm$^{-3}$ only 10 and 50 % reduction in growth was noted, respectively. Similarly as in *C. vulgaris*, an increase in Ni concentration to 5 and 10 mg dm$^{-3}$ caused lethal effect. The influence of Ni on *S. bacillaris* was not so strong as compared to the other green algae and was characterized by a gradual decrease in population density with an increase in metal concentration (Fig. 8C). The greatest reduction in growth, 80 % in relation to the control, was noted at 10 mg dm$^{-3}$. Different results were obtained in the case of a diatom, *C. meneghiniana*. In the concentrations ranging from 0.1 to 5 mg dm$^{-3}$ Ni strongly promoted the algal growth (Fig. 8D). The highest cell number (~220 % as compared to the control) was observed at 1 mg dm$^{-3}$. The population density was almost 5 times lower than in the control culture only at 10 mg Ni dm$^{-3}$.

**Lead**

The presence of lead in *O. submarina, S. bacillaris* and *C. meneghiniana* cultures caused a gradual decrease in the cell number with an increase in metal.
Fig. 9. Cell number after 9 days of culture at different Pb concentration. A – D as in Fig. 4

concentration. The lowest concentration, 0.1 mg dm$^{-3}$ Pb, did not affect the cell number in *C. meneghiniana* (rcy. 9D), but inhibited the growth of *O. submarina* (Fig. 9B) and *S. bacillaris* (Fig. 9C) to 80 and 70 % as compared to the control, respectively. On the other hand, the highest concentration (10 mg dm$^{-3}$) significantly reduced the population densities. In *S. bacillaris*, *C. meneghiniana* and *O. submarina* the reduction in cell number as compared to the control was 25, 10 and 5%, respectively. In *C. vulgaris* a somewhat different response was found. The concentrations ranging from 0.1 to 0.5 mg dm$^{-3}$ caused a slight increase in the growth of culture (Fig. 9A). Higher concentrations inhibited the algal growth. At the highest concentration tested the cell number was almost 10 times lower than in control.

**Manganese**

The influence of manganese on the growth of green algae and diatom is quite different. In green algae an increase in Mn concentration caused a gradual decrease in the growth of cultures. In *C. vulgaris* (Fig. 10A) population density steadily decreased with the increase in Mn concentration and at the highest concentration (10 mg dm$^{-3}$) the cell number was about 35 % as compared to the control. Similar results were noted in *O. submarina* (Fig. 10B) and
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Fig. 10. Cell number after 9 days of culture at different Mn concentration. A – D as in Fig. 4. Different scale in section D

*S. bacillaris* (Fig. 10C), but a marked reduction in growth was observed only at higher Mn concentrations, 5 and 10 mg dm⁻³. A different response was found in *C. meneghiniana*. In all concentrations tested there was no inhibiting influence of manganese on diatom growth and only growth promoting effect was observed (Fig. 10D), especially strong in the range from 1 to 10 mg dm⁻³.

**Iron**

The influence of iron was very similar in *O. submarina* (Fig. 11B) and *S. bacillaris* (Fig. 11C). In the range from 0.1 to 1 mg dm⁻³ iron did not have a significant effect on the cell number of the both species. At 5 and 10 mg dm⁻³ it caused a slight, about 20 % reduction in algal growth. *C. vulgaris* showed different response to iron (Fig. 11A). At 0.1 and 0.5 mg dm⁻³ the cell number was increased by a factor of 2 and 1.5 in relation to the control, respectively. At higher concentrations, 1–10 mg dm⁻³, about 40 % reduction in algal growth was observed. A quite different reaction to iron was noted in the diatom, *C. meneghiniana*. A strong stimulation of algal growth was found in all concentrations tested (Fig. 11D). The highest cell density, twice as large as in control, was noted at 5 mg dm⁻³.
Fig. 11. Cell number after 9 days of culture at different Fe concentration. A – D as in Fig. 4

4. Discussion

Even when the concentration of metal cations in the environment is very low, phytoplankton could take up and accumulate them in their cells (Davies 1978; Subba Rao 1981; Sundah 1990). This ability applies to the metals necessary to normal growth and development as well as to those of unknown metabolic function (Sorrentino 1978). Increased concentrations of all heavy metals show similar inhibiting influence on algae and this effect is intensified with an increase in metal concentration (Coleman et al. 1971). It results in growth rate reduction, morphological changes or cell death. The main parameters used in estimation of harmful effects of heavy metals on algae are: fresh or dry biomass, cell number, photosynthesis and respiration rates, concentrations of chlorophylls and other pigments and ATP, DNA and RNA levels (Sorrentino 1978).

In the present work the effects of heavy metals influence were determined from the cell number measurements of algae cultivated in laboratory conditions and the values of NOEC, EC_{10}, EC_{50} and TGI were calculated. The results obtained are summarized in Table I. On the base of the data obtained three