Is iron a limiting factor of *Nodularia spumigena* blooms?*

OCEANOLOGIA, 45 (4), 2003.
pp. 679–692.

KEYWORDS
Nodularia spumigena
Iron
Growth
Bloom
Baltic Sea

Lidia Paczuska
Alicja Kosakowska

Institute of Oceanology,
Polish Academy of Sciences,
Powstańców Warszawy 55, PL–81–712 Sopot, Poland;

e-mail: lipa@iopan.gda.pl

Manuscript received 12 September 2003, reviewed 24 October 2003, accepted 27 October 2003.

Abstract

It is well known that a deficiency of iron, a trace element essential to every living organism, limits the growth of algae and cyanobacteria. *Nodularia spumigena* Mertens is a blue-green algae species inhabiting the Baltic region that often forms toxic blooms.

The aim of the study was to assess the growth of the toxic cyanobacteria with respect to iron bioavailability. The measured growth parameters were the numbers of cells (optical density), chlorophyll *a* and pheopigment *a* concentrations. The iron concentrations used ranged from 10^{-7} to 10^{-4} mol dm^{-3}. Under iron stress conditions ($< 5 \times 10^{-7}$ mol dm^{-3}), growth inhibition, gradual pigment decay and cell mortality were observed. However, enriching the medium with complexing factors like citric acid and EDTA significantly stimulated the growth rate and chlorophyll *a* production. The citric acid – EDTA – Fe (5×10^{-7} mol dm^{-3}) complex was demonstrably effective in stimulating the rate of cell division. Starting with 10^{-6} mol dm^{-3}, the higher the iron(III) concentration used in the media, the more intensive the growth of the cyanobacteria populations. This was most rapid

* The study was carried out as part of the statutory activities of the Institute of Oceanology PAS (grant No. II.3/2003) and was supported by the Polish State Committee for Scientific Research (grant No. 3 PO4F 059 23).

in the presence of high iron concentrations (10^{-4} mol dm^{-3}), regardless of the presence of complexing agents.

It appears that the growth of toxic cyanobacteria *N. spumigena*, and thus also its ability to form blooms, may well depend on iron availability in the environment.

1. Introduction

Nodularia spumigena blooms are frequent summer occurrences in the Baltic Sea. Although such blooms have been recorded in the region since the late 19th century (Horstman 1975, Niemi 1979), during the 20th the Baltic Sea received a mass input of nutrients (Larsson et al. 1985) and blooms became more intense than ever (Kahru et al. 1994). In general, warm weather conditions, a neutral or slightly alkaline pH, light winds, and a low N:P ratio are thought to promote cyanobacterial (*Nodularia* spp. and *Aphanizomenon* spp.) blooms in the Baltic (Kononen & Niemi 1984). *N. spumigena* warrants special attention as it has the ability to produce toxic nodularin (Rinehart et al. 1988, Sivonen et al. 1989, Codd et al. 1999, Mazur & Pliński 2003), a hepatotoxin that is a proven cause of cancer of the liver (Ohta et al. 1994, Fujiki et al. 1996).

A key element in essential biological processes, iron is required for the growth of every phytoplankton cell. It is necessary for the synthesis of the chlorophyll precursor – δ-amino-levulinic acid (Marsh et al. 1963). It is also a co-factor in cytochromes and enzymes like nitrogenase and nitrate reductase in nitrogen-fixing phytoplankton such as heterocystous *Nodularia* sp. Important anti-oxidant enzymes like peroxidase and catalase also contain iron(III) (Stryer 1997).

The lack of iron in a cell inhibits growth, nitrogen fixation and enzymatic processes, and also leads to the modification of the photosynthetic pigment composition (Rueter et al. 1990, Trick et al. 1995, Wilhelm et al. 1996, Kosakowska 1999). Öquist (1971, 1974) was one of the first researchers to demonstrate specific changes to pigments under iron stress conditions.

In situ experiments, e.g. iron enrichment experiments performed in the equatorial Pacific and the Southern Ocean, have demonstrated the effect of iron on phytoplankton, (Martin et al. 1994, Coale et al. 1996). Since the metal is believed to be a growth-limiting factor in cyanobacteria also in the Baltic (Stal et al. 1999), its influence on toxic, bloom-forming species needs to be determined. Up till now, no experiments have been conducted on the influence of iron on *N. spumigena*.

Although iron is the fourth most abundant element in the Earth's crust, it is present only in ultra-trace concentrations in seawater. The fact that the concentration of iron in coastal waters (riverine and aeolian dust input)

is usually high does not guarantee the metal's availability for phytoplankton (Öztürk et al. 2002).

The oxidation-reduction chemistry of Fe(III) to Fe(II) plays a major role in iron geochemistry and biology. According to Johnson et al. (1994), the Fe(II) concentration in sea water is very low (10^{-13} M), because it is rapidly oxidised. However, Kuma et al. (1992) reported that during algal blooms in an estuarine environment, 15–20% of the total dissolved Fe was Fe(II) owing to reduction by organic substances phytoplanktonic origin. Under aerobic conditions, iron occurs in the Fe(III) state, but its inorganic solubility is very low. Moreover, its chemical speciation, and thus its biological availability, depends on the existence of dissolved organic ligands and the presence of light. About 99% of the dissolved iron in surface waters is considered to be bound to organic ligands which exist in far greater concentrations than iron itself (Gledhill & van den Berg 1994). On the one hand, the fact that the iron is bound to organic ligands retains the metal in the water column; on the other, this makes less of it available for utilisation by phytoplankton, unless they are siderophore-like substances produced by microorganisms and phytoplankton to obtain iron (Murphy et al. 1976, Simpson & Neilands 1976, Armstrong & Van Baalen 1979, Trick 1989, van den Berg 1995, Wilhelm et al. 1996). In the Baltic Sea region siderophores of unknown origin – ferrioxamine E and G, and rhodotorulic acid – have been discovered and isolated by Kosakowska et al. (1999) and Mucha et al. (1999).

As the supply of iron in open oceanic and inshore waters creates different conditions for phytoplankton communities, these have evolved to exist in given metal concentrations. Species isolated from oceanic waters grow well at low Fe concentrations, coastal strains do not (Ryther & Kramer 1961, Brand et al. 1983, Murphy et al. 1984, Sunda et al. 1991). In addition, the Fe quotas of marine cyanobacteria species are thought to be higher than those of eukaryotic phytoplankton, probably because of the abundance of the Fe-rich photosystem I in cyanobacteria (Raven 1988, 1990) and Fe-poor cytoplasm in eukaryotes (Brand 1991).

Since EDTA has a fairly high affinity for transition metals, it was brought into use as a trace metal buffering reagent for phytoplankton cultures by Hutner et al. (1950) and Myers et al. (1951). Its role is to mimic the Fe-complexed forms from natural sea waters that reduce the number of free metal ions and prevent the precipitation of Fe(III). However, Fe(III)-EDTA complexes can be photodegraded to form a source of iron(II). EDTA-metal complexes are used in medicine to prevent metal ions occurring in toxic concentrations. They are also used to minimise bacterial access to food and prevent it from going bad (Lippard & Berg 1998). EDTA is

found in all sea waters, reaching them via the effluents from many different branches of industry.

Citric acid belongs to a group of hydroxamate-type siderophores. Like all siderophores, citric acid has a high affinity for iron. It is a further iron-complexing agent used in cyanobacterial culture media.

The aim of the present experiments was to investigate the influence of iron(III) in both ionic and complexed forms on the growth of the toxic Baltic cyanobacteria species *N. spumigena*.

2. Materials and Methods

2.1. Preparation of the experiments

Laboratory experiments were conducted on a xenic *Nodularia spumigena* Mertens strain isolated from the Gulf of Gdańsk and maintained in the culture collection of Gdańsk University. The medium used for the batch cultures was BG_{11} (Stanier et al. 1971) with added NaCl (6 g dm^{-3}), and its modifications with different iron concentrations. The water used for preparing the media was deionised. The medium was sterilised at 121°C for 30 minutes. The iron concentrations ranged from 5×10^{-7} to $> 10^{-4}$ mol dm^{-3} and the solutions were prepared using Titrisol ($FeCl_3$ in 15% HCl) purchased from Merck.

The iron(III) concentration in the control sample was c. $< 10^{-7}$ mol dm^{-3} (BG_{11} medium with no iron(III) and complexing agents added). Before the experiments the culture media were buffered with 1M KOH to obtain pH values of 7.5–8.0. The batch cultures were grown in light conditions of 30 ± 5 μmol m^{-2} s^{-1} with a 16h day : 8h night photoperiod. Light was measured using an SPQA 2005 spherical sensor and a Q 21859 unidirectional sensor (LiCor, USA).

Na_2EDTA and citric acid (POCH, Gliwice) were used as iron complexing agents in original medium concentrations (0.001 and 0.006 g dm^{-3}).

The test variants were:

1. S*(original composition), iron(III) concentration: 1.27×10^{-5} mol dm^{-3}
2. P**: $< 10^{-7}$ mol Fe dm^{-3} (control sample)
3. P + Fe(III) 5×10^{-7} mol dm^{-3}
4. $P_{+EDTA+CA}$ + Fe(III) 5×10^{-7} mol dm^{-3}
5. P + Fe(III) 10^{-6} mol dm^{-3}
6. P + Fe(III) 5×10^{-6} mol dm^{-3}
7. P + Fe(III) 10^{-5} mol dm^{-3}

8. P + Fe(III) 10^{-4} mol dm^{-3}
9. $P_{+EDTA+CA}$ + Fe(III) 10^{-4} mol dm^{-3}

*S: BG_{11},
**P: BG_{11} medium without EDTA, citric acid and ferric-ammonium citrate,
FAC: ferric-ammonium citrate,
CA: citric acid.

To prevent contamination of the iron, all the glassware used for experiments was stored in 6M HCl for 24 hours before being rinsed with deionised water.

The inocula of *N. spumigena* were introduced to the media to obtain an OD ($\lambda = 750$ nm) value of 0.06 and a total volume of 50 cm^3. Three analyses were prepared with three replicates, each lasting two weeks.

2.2. Measurements

The growth parameters measured were optical density (OD), and the concentrations of active chlorophyll *a* and its degradation product – pheopigment *a*. OD was determined spectrophotometrically at $\lambda = 750$ nm in the course of the experiments to obtain the growth curve. The pigment content was measured once, at the end of the test.

The pigments were isolated by filtration of the cyanobacterial cultures through GF/C glass filters. The filters were then kept frozen for 24 hours, after which they were homogenised with 90% acetone. They were then stored at 4°C for 1 hour and centrifuged at 3 700 g for 20 minutes. The spectrophotometric measurements of chlorophyll *a* were made at 665 and 750 nm on a Beckman DU®-68 spectrophotometer (Parsons 1966). The pheopigment *a* was obtained by adding 60 μl of 1 M HCl per 5 ml of the extract. After 90 seconds the spectrophotometric measurements were made at the same wavelengths as for chlorophyll *a*. Lorenzen's (1967) equation was used to calculate the content of both pigments in the samples.

Means and standard deviations were calculated for all the results, and Dixon's Q test at 95% probability was performed to eliminate the uncertain ones (Zgirski & Gondko 1981).

3. Results

Under iron stress conditions, that is, BG_{11} medium devoid of EDTA, citric acid and ferric-ammonium citrate ($< 10^{-7}$ mol Fe dm^{-3}), actual growth of cyanobacteria, expressed as the optical density OD, was not observed (Fig. 1). Growth inhibition, decaying pigments and cell lysis were observed from the beginning of the test. Also the highest pheopigment

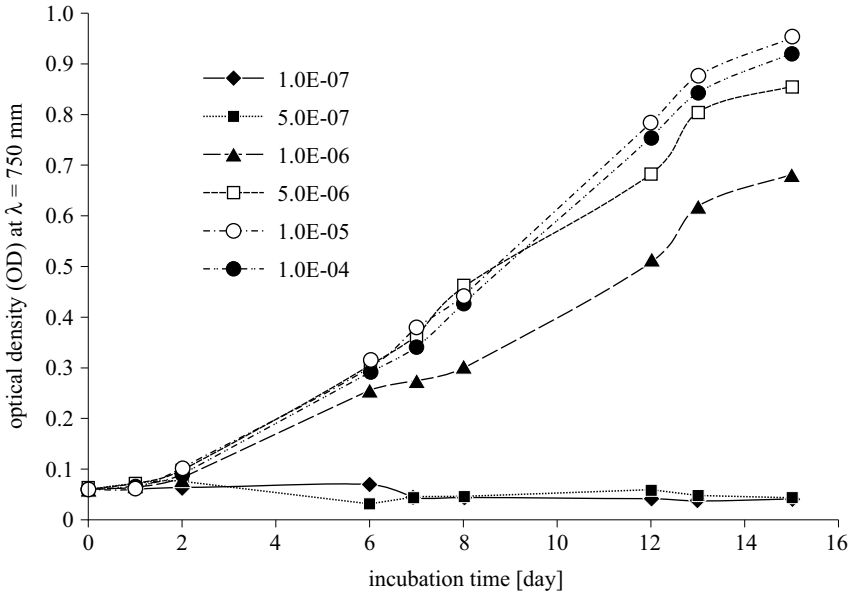

Fig. 1. Growth curves of *Nodularia spumigena* cultures at different iron(III) concentrations. The results are the means of nine replicates. Control: P – BG_{11} medium devoid of iron and iron-complexing agents. The iron(III) concentration in the control sample was c. 10^{-7} mol dm^{-3}

concentration (1.2 mg dm^{-3}) was noted under these conditions, which exceeded the content of active chlorophyll *a*. With a 5-times greater iron(III) concentration the effects were similar (Table 1, Fig. 2).

Before the experiments, the cultures of *N. spumigena* were not grown in an iron-deficient medium as in such a medium the cells died within 3–5 days of incubation.

Increasing the concentration of ionic iron to 10^{-6} mol dm^{-3} caused an increase in growth visible already after two days of experiment. At iron(III) concentrations $> 10^{-6}$ mol dm^{-3} (5×10^{-6}, 10^{-5}, 10^{-4} mol dm^{-3}) OD indicated the most intense growth of the populations (Fig. 1). Similarly, the concentrations of chlorophyll *a* were highest in these populations: 8.32–9.25 mg dm^{-3} (Table 1, Fig. 2).

Interestingly, enrichment of an iron-poor medium (5×10^{-7} mol Fe dm^{-3}) with iron complexing factors – EDTA and citric acid – accelerated the growth rate and chlorophyll *a* production by a factor of one hundred (Table 2, Fig. 3). Under iron-rich conditions (10^{-4} mol dm^{-3}) the addition of complexing factors did not have any significant effects on the growth of *N. spumigena* (Table 2, Fig. 3). By comparison, an experiment

Table 1. Chlorophyll *a* and pheopigment content in *Nodularia spumigena* cultures from different concentrations of ionic iron(III). The data are the means of 9 replicates ±SD. Control: P – BG_{11} medium devoid of iron and iron-complexing agents. The iron(III) concentration in the control sample was c. 10^{-7} mol dm^{-3}

iron(III) concentrations [mol dm^{-3}]	Concentration of pigments [mg dm^{-3}] × ±SD	
	chlorophyll *a*	pheopigment
$< 10^{-7}$ (control)	0.19 ± 0.00	1.20 ± 0.00
5×10^{-7}	0.13 ± 0.00	0.20 ± 0.00
10^{-6}	6.44 ± 0.83	0.17 ± 0.03
5×10^{-6}	9.32 ± 1.24	0.56 ± 0.19
10^{-5}	9.84 ± 1.12	0.67 ± 0.08
10^{-4}	9.25 ± 1.23	0.51 ± 0.20

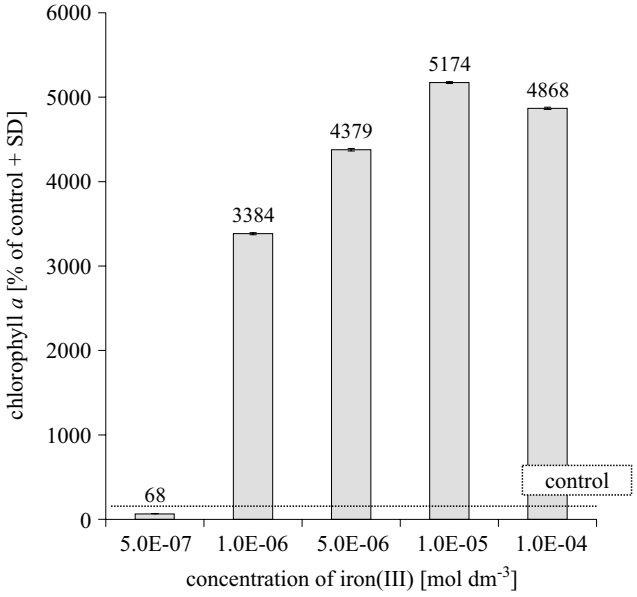

Fig. 2. Influence of the ionic form of iron(III) on chlorophyll *a* concentrations in *Nodularia spumigena* cultures. The results are the means of 9 replicates. Control: P – BG_{11} medium devoid of iron and iron-complexing agents. The iron(III) concentration in the control sample was c. 10^{-7} mol dm^{-3}

Table 2. Chlorophyll *a* content in *Nodularia spumigena* cultures grown in media with different forms of iron(III). The data are the means of nine replicates ±SD

iron(III) concentrations [mol dm^{-3}]	Concentration of pigments [mg dm^{-3}] × ±SD	
	ionic iron(III)	complexed iron(III) EDTA, CA
5×10^{-7}	0.03 ± 0.02	3.11 ± 0.38
10^{-4}	7.76 ± 1.40	7.24 ± 3.15

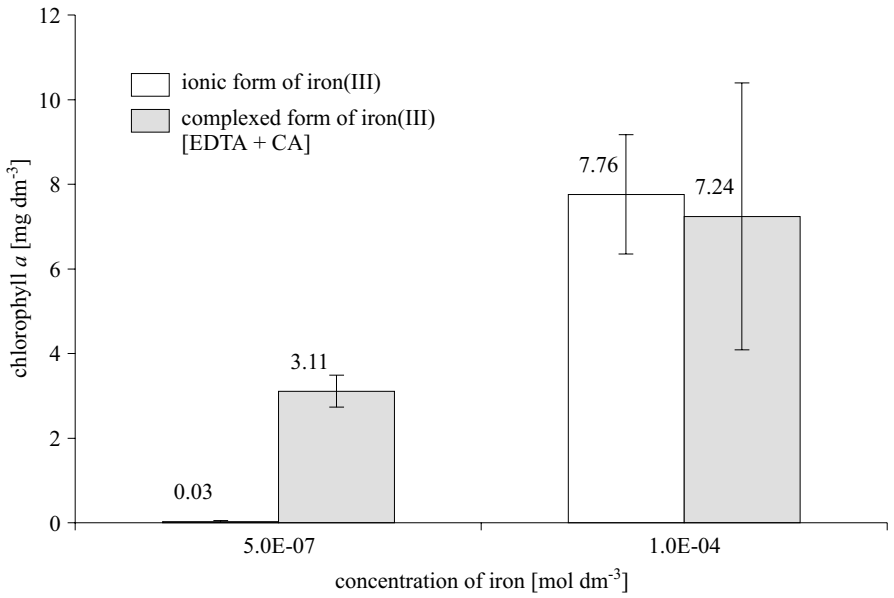

Fig. 3. Chlorophyll *a* concentrations in *Nodularia spumigena* depending on the form of iron(III). The results are the means of nine replicates

on standard BG_{11} medium with iron(III) ions complexed with EDTA and CA displayed optimal growth conditions with high chlorophyll *a* concentrations of 11.28 mg dm^{-3} and concentration of pheopigment *a* 0.31 mg dm^{-3} (Fig. 4).

4. Discussion

The above experiments clearly demonstrate that growth of the Baltic cyanobacteria species *N. spumigena* is related to the concentration of iron

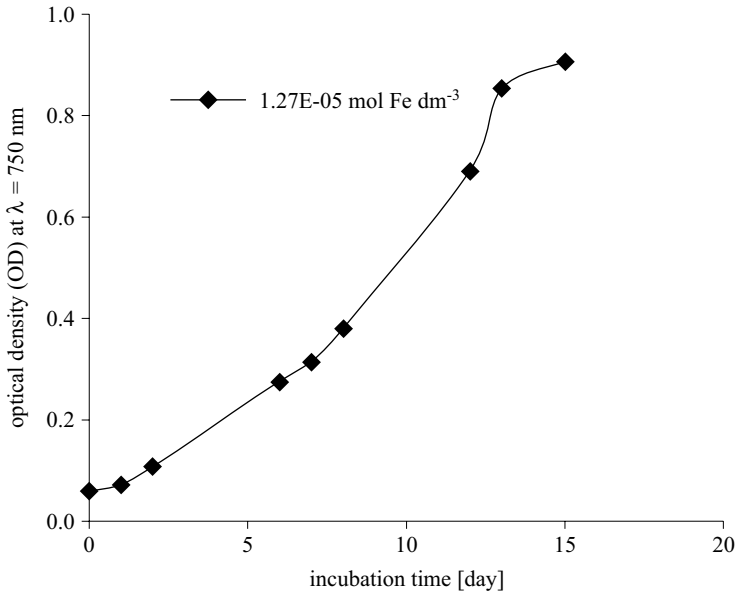

Fig. 4. Optical density (OD) in a population of *Nodularia spumigena* incubated in BG_{11} medium

in the environment. At iron(III) concentrations of 5×10^{-7} mol dm^{-3} and less, growth of these populations was impossible, and cell mortality was distinct, considering the high pheopigment *a* content. Clearly, these iron-limited conditions are insufficient for chlorophyll *a* production and enzymatic processes to take place in the cells. It is also possible that the physico-chemical form of the metal was unavailable to the *N. spumigena* population.

Starting with an iron concentration of 10^{-6} mol dm^{-3}, cyanobacterial growth was observed and intensified with increasing metal concentration, up to 10^{-4} mol Fe dm^{-3}. A similar tendency was noted in experiments on *Oscillatoria tenuis* (Brown & Trick 1992, Trick et al. 1995). The cyanobacterium was unable to grow well at iron(III) concentrations of 4.1×10^{-7}, and the highest chlorophyll *a* concentration was observed at 4.7×10^{-6} mol Fe dm^{-3}. Furthermore, in research carried out on *Synechocystis aquatilis* and *Anabaena variabilis*, the stimulatory influence of raising the iron(III) concentration was noted in the concentration range of 10^{-8} to 5×10^{-5} mol Fe dm^{-3}. The highest quantity of chlorophyll *a* was attained at an iron concentration of 2 to 5×10^{-5} mol dm^{-3}. However, any further increase in iron concentration led to an inhibition of growth, and this ceased altogether once concentrations of 10^{-3} mol Fe dm^{-3} were reached (Surosz et al. 1994, Kosakowska 1999). Likewise, in experiments performed

on another blue-green algae species – *Synechococcus* sp. PCC 7002 – there was an obvious inhibition of growth with falling iron(III) concentration, from 4.7×10^{-6} to 3.1×10^{-8} mol dm^{-3}. Interestingly, a further reduction in the iron(III) content in the media was followed by accelerated growth of the culture resulting from the production of siderophores (Wilhelm et al. 1996).

At high iron concentrations (10^{-4} mol dm^{-3}) neither form of the metal was preferred by *N. spumigena*, that is to say, the cyanobacteria grew equally well in the presence of the ionic and complexed forms of iron (Table 2, Fig. 3). Soria-Dengg et al. (2001) obtained similar results in experiments on different forms of ferric complexes. At high iron concentrations, the growth rate of the diatom *Phaeodactylum tricornutum* was not influenced by the form of iron in the medium.

A discussion has been held on the use of EDTA to mimic iron complexation in culture experiments (Gerringa et al. 2000). Our results show that the presence of the EDTA-citric acid complex in the culture media enhanced cyanobacteria growth at low iron concentrations. The EDTA-Fe complex appeared to be an effective and stable iron (III) source for *N. spumigena* under conditions of stress. However, when the iron(III) concentration was high (10^{-4} mol dm^{-3}), the cyanobacteria were able to use either the ionic or the complexed form of the metal.

The bioavailability of iron in the sea is evidently influenced by bacteria that produce siderophores, not only for their own use, but also for phytoplankton lacking this ability (Soria-Dengg & Horstmann 1995). The above experiments have demonstrated that the growth of *N. spumigena* under iron-limited conditions (5×10^{-7} mol dm^{-3} or less) was strongly inhibited and that cells began to die within few days. Cultures were therefore not pre-incubated under metal deficiency conditions, as some other authors have suggested (Trick et al. 1995, Wilhelm et al. 1996, Kosakowska et al. 2004). It seems that the cyanobacteria strain examined in the experiments is unable to produce siderophores by itself, but can probably utilise the existing ones of bacteriological origin.

Our results show that the growth, and thus, very likely, the bloom forming ability of *N. spumigena* too, are dependent on iron(III) bioavailability. The large-scale dilution of riverine loads is thought to be primarily responsible for the trace metal distribution in the Gulf of Gdańsk (Pempkowiak et al. 2000). Owing to the large quantities of iron carried to the sea by rivers and the atmosphere, coastal regions are regarded as iron-sufficient. As Rydin et al. (2002) reported, the concentration of bioavailable iron in the Baltic of > 20 μg dm^{-3} is high enough to allow *N. spumigena* and other diazotrophic cyanobacteria like *Aphanizomenon* spp. to bloom, despite their probably high iron demands.

The iron(III) concentration appears to be a further factor contributing to *N. spumigena* blooms in the Baltic Sea.

Acknowledgements

The authors wish to thank Dr H. Mazur-Marzec and Prof. M. Pliński for the *Nodularia spumigena* strain, the subject of the study.

References

Armstrong J. E., Van Baalen C., 1979, *Iron transport in microalgae: the isolation and biological activity of a hydroxamate siderophore from the blue-green algae Agmenellum quadruplicatum*, J. Gen. Microbiol., 111, 253–262.

Berg C. M. G. van den, 1995, *Evidence for organic complexation of iron in seawater*, Mar. Chem., 50, 139–157.

Brand L. E., 1991, *Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production*, Limnol. Oceanogr., 36 (8), 1756–1771.

Brand L. E., Sunda W. G., Guillard R. R. L., 1983, *Limitation of marine phytoplankton reproductive rates by zinc, manganese and iron*, Limnol. Oceanogr., 28, 1182–1198.

Brown C. M., Trick C. G., 1992, *Response of the cyanobacterium, Oscillatoria tenuis, to low iron environments: the effect on the growth rate and evidence for siderophore production*, Arch. Microbiol., 157, 349–354.

Coale K. H., Johnson K. S., Fitzwater S. E., Gordon R. M., Tanner S., Chavez F. P., Ferioli L., Sakamoto C., Rogers P., Millero F., Steinberg P., Nightingale P., Copper D., Cochlan W. P., Landry M. R., Constantinou J., Rollwagen G., Trasvina A., Kudela R., 1996, *A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean*, Nature, 383, 495–501.

Codd G. A., Bell S. G., Kaya K., Ward C. J., Beattie K. A., Metcalf J. S., 1999, *Cyanobacterial toxins, exposure routes and human health*, Eur. J. Phycol., 34, 405–415.

Fujiki H., Sueke E., Suganuma M., 1996, *Carcinogenesis of microcystins*, [in:] *Toxic microcystis*, M. F. Watanabe, K. I. Harada, W. W. Carmichael & H. Fujiki (eds.), CRC Press, Boca Raton, 252 pp.

Gerringa L. J. A., de Baar H. J. W., Timmermans K. R., 2000, *A comparison of iron limitation of phytoplankton in natural oceanic waters and laboratory media conditioned with EDTA*, Mar. Chem., 68, 335–346.

Gledhill M., Van den Berg C. M. G., 1994, *The determination of complexation of iron(III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry*, Mar. Chem., 47, 41–54.

Horstmann U., 1975, *Eutrophication and mass production of blue-green algae in the Baltic*, Merentutkimuslait. Julk./Havsforskningsinst. Skr., 239, 83–90.

Hutner S. H., Provasoli L., Schatz A., Haskins C. P., 1950, *Some approaches to the study of the role of metals in the metabolism of microorganisms*, Proc. Am. Philos. Soc., 94, 152–170.

Johnson K. S., Coale H. K., Elrod V. A., Tindale N. W., 1994, *Iron photochemistry in seawater from the equatorial Pacific*, Mar. Chem., 46, 319–334.

Kahru M., Horstmann U., Rud O., 1994, *Satellite detection of increased cyanobacterial blooms in the Baltic Sea: natural fluctuation or ecosystem change?*, Ambio, 23 (8) 469–472.

Kononen K., Niemi Å., 1984, *Long-term variation of the phytoplankton composition at the entrance to the Gulf of Finland*, Ophelia, Suppl. 3, 101–110.

Kosakowska A., 1999, *The influence of iron and selected organic compounds on Baltic Sea phytoplankton, (Habilitation thesis in ecophysiology and biochemistry of marine algae)*, Rozpr. i monogr., Inst. Oceanol. PAN, Sopot, 11, 73–75, (in Polish with English summary).

Kosakowska A., Kupryszewski G., Mucha P., Rekowski P., Lewandowska J., Pazdro K., 1999, *Identification of selected siderophores in the Baltic Sea environment by the use of capillary electrophoresis*, Oceanologia, 41 (4), 573–587.

Kosakowska A., Lewandowska J., Stoń J., Burkiewicz K., 2004, *Qualitative and quantitative composition of pigments in Phaeodactylum tricornutum (Bacillariophyceae) stressed by iron*, BioMetals, 17, 45–52.

Kuma K., Nakabayashi S., Suzuki Y., Kudo I., Matsunaga K., 1992, *Photoreduction of Fe(III) by dissolved organic substances and existence of Fe(II) in seawater during spring blooms*, Mar. Chem., 37, 15–27.

Larsson U., Elmgren R., Wulff F., 1985, *Eutrophication and the Baltic Sea: causes and consequences*, Ambio, 14 (1), 9–14.

Lippard S. J., Berg J. M., 1998, *Podstawy chemii bionieorganicznej*, PWN, Warszawa, 36 pp.

Lorenzen S. J., 1967, *Determination of chlorophyll and pheopigments: spectrophotometric equations*, Limnol. Oceanogr., 12, 343–346.

Marsh H. V., Evans H. J., Matrone G., 1963, Plant Physiol., 38 (6), pp. 632–638; 638–642, [after citation in:] *Udział mikroelementów w metabolizmie roślin*, PWRiL, Warszawa, 1971, 160 pp., (the Polish translation by Z. Sójkowski).

Martin J. H., Coale K. H., Johnson K. S., Fitzwater S. E., Gordon R. M., Tanner S. J., Hunter C. N., Elrod V. A., Nowicki J. L., Coley T. L., Barber R. T., Lindley S., Watson A. J., Van Scoy K., Law C. S., Liddicoat M. I., Ling R., Stanton T., Stockel J., Collins C., Anderson A., Bidigare R., Ondrusek M., Latasa M., Millero F. J., Lee K., Yao W., Zhang J. Z., Friederich G., Sakamoto C., Chavez F. P., Buck K., Kolber Z., Greene R., Falkowski P., Chisholm S. W., Hoge F., Swift R., Yungel J., Turner S., Nightingale P., Hatton A., Liss P., Tindale N. W., 1994, *Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean*, Nature, 37, 123–129.

Mazur H., Pliński M., 2003, *Nodularia spumigena blooms and the occurrence of hepatotoxin in the Gulf of Gdańsk*, Oceanologia, 45 (2), 305–316.

Mucha P., Rekowski P., Kosakowska A., Kupryszewski G., 1999, *Separation of siderophores by capillary electrophoresis*, J. Chromatogr., 830 (A), 183–189.

Murphy L. S., Guillard R. R. L., Brown J. F., 1984, *The effects of iron and manganese on copper sensitivity in diatoms: differences in the responses of closely related neritic and oceanic species*, Biol. Oceanogr., 3, 187–201.

Murphy T. P., Lean D. R. S., Nalewajko C., 1976, *Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae*, Science, 192, 900–902.

Myers J., Philips J. N., Jr., Graham J.–R., 1951, *On the mass culture of algae*, Plant Physiol., 26, 539–548.

Niemi A., 1979, *Blue-green algal blooms and N:P ratio in the Baltic Sea*, Acta Bot. Fenn., 110, 57–61.

Ohta T., Sueoka E., Iida N., Komori A., Suganuma M., Nishiwaki R., Tatematsu M., Kim S. J., Carmichael W. W., Fujiki H., 1994, *Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver*, Cancer Res., 54, 6402–6407.

Öquist G., 1971, *Changes in plant composition and photosynthesis induced by iron-deficiency in the blue-green alga Anacystis nidulans*, Plant Physiol., 25, 188–191.

Öquist G., 1974, *Iron deficiency in the blue-green alga Anacystis nidulans: changes in pigmentation and photosynthesis*, Plant Physiol., 30, 30–37.

Öztürk M., Steinnes E., Sakshaug E., 2002, *Iron speciation in the Trondheim Fjord from the perspective of iron limitation for phytoplankton*, Estuar. Coast. Shelf Sci., 55, 197–212.

Parsons T. R., 1966, *The determination of photosynthetic pigments in sea-water. A survey of methods*, [in:] *Determination of photosynthetic pigments in sea-water*, SCOR–UNESCO, Paris.

Pempkowiak J., Chiffoleau J.-P., Staniszewski A., 2000, *Vertical and horizontal distribution of selected heavy metals in the Southern Baltic Sea off Poland*, Estuar. Coast. Shelf Sci., 51 (1), 115–125.

Raven J. A., 1988, *The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources*, New Phytol., 109, 279–287.

Raven J. A., 1990, *Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway*, New Phytol., 116, 1–18.

Rinehart K. L., Harada K. I., Namikoshi M., Chen C., Harvis C. A., Munro M. H., Blunt J. W., Muligan P. E., Beasley V. R., Dahlem A. M., Carmichael W. W., 1988, *Nodularin, microcystin, and the configuration of ADDA*, J. Am. Chem. Soc., 110 (25), 8557–8558.

Rueter J. G., Ohki K., Fujita Y., 1990, *The effect of iron nutrition on photosynthesis and nitrogen fixation in cultures of Trichodesmium (Cyanophyceae)*, J. Phycol., 26, 30–35.

Rydin E., Hyenstrand P., Gunnerhed M., Blomqvist P., 2002, *Nutrient limitation of cyanobacterial blooms: an enclosure experiment from the coastal zone of the northwest Baltic proper*, Mar. Ecol. Prog. Ser., 239, 31–36.

Ryther J. H., Kramer D. D., 1961, *Relative iron requirement of some coastal and offshore plankton algae*, Ecology, 42, 444–446.

Simpson F. B., Neilands J. B., 1976, *Siderochromes in cyanophyceae: isolation and characterization of schizokinen from Anabaena sp.*, J. Phycol., 12, 44–48.

Sivonen K., Kononen K., Esala A. L., Niemelä S. I., 1989, *Toxicity and isolation of the cyanobacterium Nodularia spumigena from the southern Baltic Sea in 1986*, Hydrobiologia, 185, 3–8.

Soria-Dengg S., Horstmann U., 1995, *Ferrioxamine B and E as iron source for the marine diatom Phaeodactylum tricornutum*, Mar. Ecol. Prog. Ser., 127, 269–277.

Soria-Dengg S., Reissbrodt R., Horstmann U., 2001, *Siderophores in marine coastal waters and their relevance for iron uptake by phytoplankton: experiments with the diatom Phaeodactylum tricornutum*, Mar. Ecol. Prog. Ser., 220, 73–82.

Stal L. J., Staal M., Villbrandt M., 1999, *Nutrient control of cyanobacterial blooms in the Baltic Sea*, Aquat. Microbiol. Ecol., 18, 165–173.

Stanier R. Y., Kunisawa R., Mandel M., Cohen-Bazire G., 1971, *Purification and properties of unicellular blue-green algae (Order Chroococcales)*, Bact. Rev., 35, 171–205.

Stryer L., 1997, *Biochemia*, PWN, Warszawa, 1132 pp.

Sunda W. G., Swift D. G., Huntsman S. A., 1991, *Low iron requirement for growth in oceanic phytoplankton*, Nature, 351, 55–57.

Surosz W., Kosakowska A., Falkowski L., 1994, *Wpływ żelaza na zawartość chlorofilu a i inkoroprację węgla-14 w kulturach glonów Chlorella vulgaris Beijerinck i Anabaena variabilis Kützing*, Zesz. Nauk. Uniw. Gdańsk., Oceanografia, 13, 71–87.

Trick C. G., 1989, *Hydroxamate-siderophore production and utilization by marine eubacteria*, Curr. Microbiol., 18, 375–378.

Trick C. G., Wilhelm S. W., Brown C. M., 1995, *Alterations in cell pigmentation, protein expression and photosynthetic capacity of the cyanobacterium Oscillatoria tenuis, grown under low iron conditions*, Can. J. Microbiol., 41, 117–123.

Wilhelm S. W., Maxwell D. P., Trick C. G., 1996, *Growth, iron requirements and sideophore production in iron-limited Synechococcus PCC 7002*, Limnol. Oceanogr., 41 (1), 89–97.

Zgirski A., Gondko R., 1981, *Obliczenia biochemiczne*, PWN, Warszawa, 4–45.