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THE INFLUENCE OF GIBBERELLIC ACID ON THE IRON UPTAKE BY THE BALTIC PHYTOPLANKTON

Contents: 1. Introduction, 2. Materials and methods, 3. Results and discussion; Streszczenie; References.

The influence of gibberellic acid (GA_3) upon the iron uptake and primary production in Baltic phytoplankton and in standard phytoplankton cultures, grown on synthetic media and natural sea water, has been investigated.

Iron uptake was measured by radio isotope technique. The primary production and biomass were examined by carbon isotope method and by the determination of chlorophyll respectively.

In synthetic media GA_3 markedly increased Fe uptake by phytoplankton but only at the restricted range of pH 6—6,5. Most probably this stimulation is due to the formation of assimilable Ga_3-iron complex. The growth stimulation effect of GA_3 results probably from the increased uptake of iron by phytoplankton.

In natural sea water GA_3-iron mixture, depending on the time and place of sampling, stimulates inhibits or has no influence on phytosynthesis and biomass production. The possible factors interfering with GA_3 action in natural environments have been discussed.

1. INTRODUCTION

Phytoplankton gowth in sea water is often restricted by the lowered availability of some trace elements, the uptake of which depends on many factors [8, 13]. Especially important for phytoplankton development is iron (9) but, there exist only little informations about its distribution in the Baltic sea [2, 3]. It was found that the amount of iron in Baltic sea water is markadly lower than that used for the cultivation of phytoplankton [1, 13]. The effective concentration of sea water iron is still lower than its total contents because it appears in various chemical forms and only some of them are assimilable for phytoplankton. Iron is assimilated by algae as ferric ions most probably complexed with some natural organic compounds contained in sea water (so called siderochromes). The ratio of assimilable and nonassimilable iron in Baltic sea water has not yet been established.

The rather high contents of iron in phytoplankton cells, in regard to very low concentration of assimilable iron in the sea waters, is obtained in the result of cellular active accumulation processes against the concentration gradients. The factors facilitating such an accumulation play an important role in the development of phytoplankton.

We have observed that gibberellic acid, an important plant growth regulator [11, 12] found also in Baltic waters (Kentzer, unpublished data), markedly stimulates the development of phytoplankton in the iron containing media [9]. The results obtained point to the enhancement of iron uptake rather than to the direct stimulation of metabolism by this plant hormone. In the present paper more detailed studies on the mechanism of the above phenomenon are presented. The influence of gibberellic acid upon the growth and iron uptake by phytoplankton has been investigated.

2. MATERIALS AND METHODS

The measurements were carried out from April till November 1974. Samples of sea water and phytoplankton were taken at ten stations in the southern Baltic (Fig. 1). Surface and bottom sea water was sampled

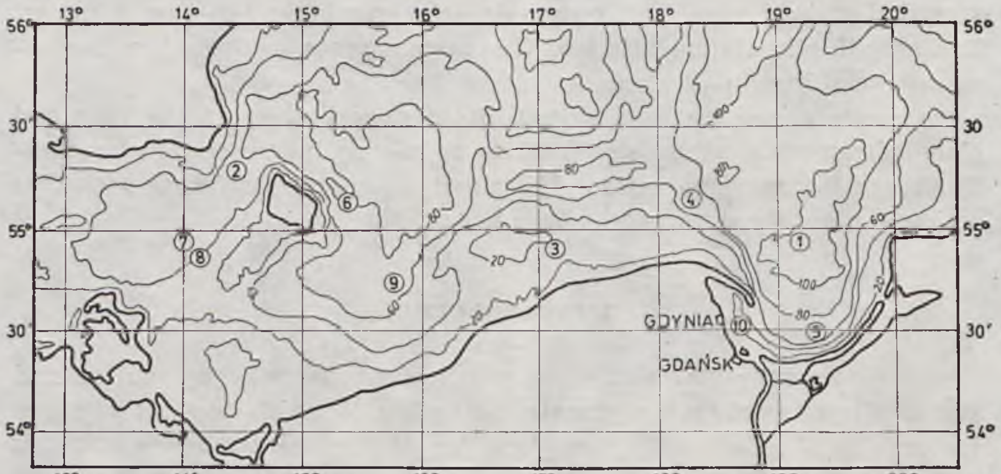

Fig. 1. Location of the sampling stations

Ryc. 1. Rozmieszczenie stacji pobrania prób by means of a polyethylene bathometer. The sea water immediately after sampling was filtered through 25 μm nylon net and afterwards through 0.45 μm Sartorius membrane filter in order to separate the suspended matter. Before the experiments the sea water was stored at low temperature (below 10°C). Some experiments were done using artificial sea water [4] and Prat's medium [1]. The suspension of phytoplankton was prepared for experiments as follows: the natural phytoplankton was collected by means of standard plankton net or by bathometer. After separating from the zooplankton by filtration through a 100 μm net and decantation from the inorganic matter, the phytoplankton was suspended to a concentration corresponding to 1 mg of dry matter in 1 ml of sea water. The phytoplankton suspension (1 ml) was added to 100 ml sea water-samples. Qualitative and quantitative analyses of phytoplankton were made for each suspension. In experiments both natural phytoplankton and some pure cultures such as Chlorella vulgaris Beijerinck and Dictyosphaerium pulchellum [1] were used. The pure cultures were used for comparative purposes and the method of their inoculation was described previously [9].

The rate of the iron uptake by the phytoplankton cells was determined by using ^{59}Fe. To 100 ml of the artificial sea water samples or to the samples of Prat's medium 1 ml of the ^{59}Fe solution was added. The radioactivity of the Fe-solution was 25 μCi/ml. In each combination one of the samples studied was enriched with 0.1 ml of gibberellic acid (GA_3) solution. The final concentration of GA_3 in these samples was 10^{-10} mM/l. The samples were incubated at optimal temperature and in light conditions. After 1 hour 10 ml portions of these samples were filtered by Sartorius membrane filters to isolate the phytoplankton cells. The remaining phytoplankton sediment was washed twice with 2 ml artificial sea water, and tested for the activity of ^{59}Fe by using the Geiger-Müller counter.

The effects of exogeneously applied GA_3 were studied in short and long-term experiments. The results of short action of GA_3 were estimated by measuring the primary production after 4 hours of phytoplankton incubation. The carbon isotope ^{14}C method developed by Steemann Nielsen was used for measurements of the primary production [7, 14]. The samples of the phytoplankton suspended in the sea water enriched with a gibberellin-Fe mixture (0.02 mM Fe/l 1 and 1×10^{-7} mM GA_3/l) were incubated in the illuminator at a constant temperature for 4 hours. The temperature was kept at the same value as it had been found in the sea at the time the phytoplankton was collected. For comparison the primary production of three control samples was investigated at the same time and the same conditions:

I — for the phytoplankton suspended in the sea water enriched with 0.02 mM Fe/l.

II — for the phytoplankton suspended in the sea water enriched with 1×10^{-7} mM GA_3/l.

III — for the phytoplankton suspended in "pure" sea water.

In all experiments the phytoplankton samples were incubated in continuous light with intensity of about 4000 lux.

The long-term influence of GA_3 on the biomass production of the phytoplankton was measured by estimation of the contents of the photosynthetic pigment — chlorophyll a, after 8 days of incubation. Samples of 400 ml sea water containing the phytoplankton suspension and the control samples were prepared as described above, but the amounts of gibberellic acid and iron used were higher in this case. 6×10^{-7} mM/l of GA_3 and 0.05 mM/l of iron were added. In addition the water samples were enriched with nutrients solution in amounts required for the optimal growth during a long-term incubation [6]. The samples were earated for 12 hours a day and illuminated continously. After incubation analyses of chlorophylls and carotenoids were made [15]. The filtering procedure and analyses of the photosynthetic pigments were carried out according to Calberg and Gargas [5, 7]. The phytoplankton biomass was measured as dry matter.

3. RESULTS AND DISCUSSION

Table 1 presents the results of the influence of exogeneously applied GA_3 on the ^{59}Fe uptake by phytoplankton in synthetic media. These experiments showed that gibberellic acid used in the very small amount of 10^{-10} mM/l was able to increase the Fe uptake by the phytoplankton markedly but only at a restricted range of the pH value of 6 or 6.5 In a neutral or alcaline medium no or negligible effects were observed.

The occurrence of stimulation only at lower pH values in all phytoplankton and media studied can not be attributed to the eventual general preference of acidic conditions for the iron uptake. Although in Pratt's medium iron accumulation by Chlorella vulgaris cells in the absence of gibberellic acid is in higher pH values slightly lower than in acidic conditions, phytoplankton I (table 2) and Dictyosphaerium pulchellum in artificial sea water accumulated iron at higher pH more than at lower.

The stimulatory action of gibberellic acid in acidic conditions could thus be explained by one of the following mechanisms. Either only a nondissociated hormone molecule is capable of traversing the cytoplasmatic membrane (or its uptake for some other reasons is possible only at low pH) and acting in a cell as general growth stimulant it increases the demand for the iron increased uptake which would be a secondary effect, or the gibberellic acid in acidic conditions (in nondissociated form?) complexes the iron ions thus facilitating their transport across the membrane by playing the role of an iron carrier (siderochrome). In the latter

Table 1
Tabela 1

The influence of gibberellic acid on the uptake of ^{59}Fe by phytoplankton
Wpływ kwasu giberelowego na pobranie ^{59}Fe przez fitoplankton

Phytoplankton Fitoplankton *	Medium Środowisko	pH of the medium pH środowiska	Uptake of ^{59}Fe (c.p.m.) Pobieranie ^{59}Fe (imp./min.)	
			A	B
C	Prat's	6.0	870	1250
		6.5	885	1190
		7.0	830	900
		7.5	750	730
		8.0	710	740
I	artificial sea water	6.0	630	980
		6.5	720	1140
		7.0	750	790
		7.5	945	920
		8.0	1020	1100
D	artificial sea water	6.0	1100	1780
		6.5	1500	1920
		7.0	1870	2010
		7.5	1740	1870
		8.0	1860	1730

* Characteristic of phytoplankton is given in Table 2.
A — phytoplankton was grown in medium enriched with ^{59}Fe.
B — phytoplankton was grown in medium enriched with ^{59}Fe and gibberellic acid.

Table 2
Tabela 2

Characteristic of phytoplankton used
Charakterystyka jakościowa fitoplanktonu

Phytoplankton Fitoplankton	Species of phytoplankton used Rodzaj badanego fitoplanktonu
I	*Chaetoceros* sp., *Diatoma elongatum*, *Navicula lacustris*, *Scenedesmus acuminatus*, *Anabaena flos-aquae*
II	*Chaetoceros borealis*, *Diatoma elongatum*, *Navicula lacustris*
III	*Euglena*, *Scenedesmus acuminatus*, *Ankistrodesmus falcatus*, *Oscillatoria* sp., *Melosira jürgensi*
IV	*Coscinodiscus grani*, *Sceletonema costatum*, *Pediastrum boryanum*
C	*Chlorella vulgaris* Beijerinck
D	*Dictyosphaerium pulchellum wood*

case the enhancement of iron uptake would be prior to the stimulation of growth resulting from the increased contents of cellular iron.

It has been demonstrated previously [10] that GA_3 in neutral or slightly acidic conditions and in the presence of iron strongly increases the phytoplankton growth but only at concentrations much higher than those used in these studies. The concentration 10^{-10} mM/l has negligible effect on growth promotion but is enough for the marked stimulation of iron uptake. Therefore it seems to be more probable that the direct influence of gibberellic acid on the iron uptake e.g. by the formation of assimilable GA_3-iron complex is the primary effect in the phenomenon of stimulation of iron accumulation by this plant hormone. The final elucidation of this problem will be a subject of our further studies.

The stimulatory effect of gibberellic acid on the iron uptake can also be helpful in studing the physiological effects of very low concentrations of GA_3 (e.g. in sea waters) on phytoplankton. In such concentrations the growth stimulation, as negligible, is not or hardly measurable. On the other hand the determinatio of radioactive iron uptake provides a useful tool in such studies.

The results presented in Table 1 indicate also the marked effect of pH itself on iron uptake by phytoplankton which probably influences the growth of these organisms. This factor might thus play a role in the development of phytoplankton in natural environments.

Apart from the demonstration of iron accumulation promoting ac-

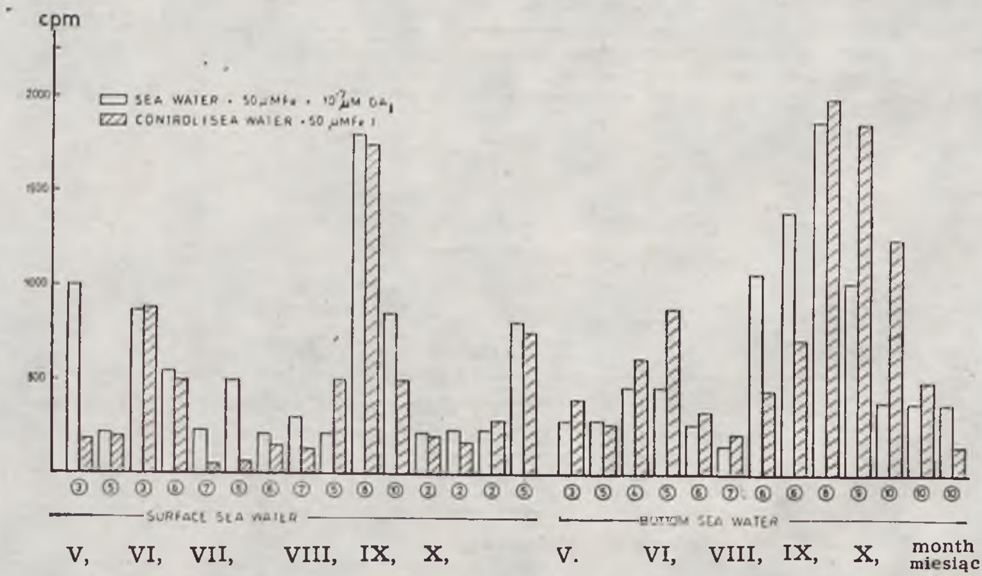

Fig. 2. The influence of GA_3 on the primary production of Baltic water enriched with iron ions. Numbers in circle represent the numbers of the various sampling stations

Ryc. 2. Wpływ GA_3 na produkcję pierwotną wód Bałtyku wzbogaconych w jony żelazowe. Numery w kółkach oznaczają punkty pobrania prób tivity by GA_3 in sythetic media the question arises whether or not such conditions which allow gibberellic acid to facilitate the iron uptake by phytoplankton occur in Baltic water. This question was the subject of some further studies.

In these experiments the short and long-term influence of the GA_3-Fe mixture added to natural Baltic water was investigated during the whole period of seasonal development of phytoplankton. The obtained results were similar in the short-. as well as in the long-term experiments. The data obtained from measuring the primary production are illustrated in Fig. 2. The effect of the long-term influence of GA_3 on the phytoplankton biomass (indexed by the contents of chlorophyll a) is shown in Tables 3 and 4. On the basis of the presented experiments it can be assumed that GA_3 can promote the development of phytoplankton (probably by the stimulation of iron uptake) also in the natural sea water conditions. Nevertheless the stimulation was not observed in all sea water

Table 3

Tabela 3

The effect of gibberellic acid, added to the surface sea water on the growth of phytoplankton expressed by the contents of chlorophyll a

Wpływ GA_3 dodanego do powierzchniowej wody morskiej na rozwój fitoplanktonu wyrażony poprzez pomiar chlorofilu a

Station Stacja pobrania prób (see Fig. 1)	Phytoplankton Fitoplankton (see Tab. 2)	Surface sea water Powierzchniowa woda morska	Contents of chlorophyll a (mg/dm^3) Zawartość chlorofilu a (mg/dm^3)
1	2	3	4
3	I	untreated	0.15
		plus GA_3	0.16
		plus Fe	0.35
		plus GA_3 and Fe	0.48
5	I	untreated	0.16
		plus GA_3	0.13
		plus Fe	0.19
		plus GA_3 and Fe	0.13
5	C	untreated	0.07
		plus GA_3	0.23
		plus Fe	0.23
		plus GA_3 and Fe	0.51
7	II	untreated	0.03
		plus GA_3	0.04
		plus Fe	0.07
		plus Fe and GA_3	0.10

1	2	3	4
5	II	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.03 0.03 0.13 0.14
6	II	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.11 0.13 0.15 0.29
7	D	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.11 0.08 0.21 0.16
5	C	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.07 0.08 0.14 0.30
8	III	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.06 0.16 0.21 0.55
10	IV	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.09 0.15 0.27 0.51
2	IV	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.16 0.16 0.20 0.38
2	D	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.12 0.13 0.15 0.24
2	C	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.12 0.11 0.14 0.25
1	IV	untreated plus GA_3 plus Fe plus GA_3 and Fe	0.68 0.21 0.82 0.24

Table 4

Tabela 4

The effect of gibberellic acid, added to the bottom sea water on the growth of phytoplankton expressed by the contents of chlorophyll a

Wpływ kwasu giberelowego dodanego do przydennej wody morskiej na wzrost fitoplanktonu obserwowany poprzez pomiar chlorofilu a

Station Stacja pobrania prób (see Fig. 1)	Phytoplankton Fitoplankton (see Tab. 2)	Bottom sea water Woda przydenna	Contents of the chlorophyll a Zawartość chlorofilu. a (mg/dm^3)
1	2	3	4
3	I	untreated	0.08
		plus GA_3	0.08
		plus Fe	0.14
		plus GA_3 and Fe	0.26
4	I	untreated	0.10
		plus GA_3	0.22
		plus Fe	0.31
		plus GA_3 and Fe	0.37
5	I	untreated	0.14
		plus GA_3	0.16
		plus Fe	0.19
		plus GA_3 and Fe	0.40
3	C	untreated	0.07
		plus GA_3	0.12
		plus Fe	0.08
		plus GA_3 and Fe	0.29
4	C	untreated	0.06
		plus GA_3	0.05
		plus Fe	0.13
		plus GA_3 and Fe	0.17
5	D	untreated	0.21
		plus GA_3	0.21
		plus Fe	0.30
		plus GA_3 and Fe	0.50
6	I	untreated	0.28
		plus GA_3	0.31
		plus Fe	0.32
		plus GA_3 and Fe	0.33

1	2	3	4
7	II	untreated	0.13
		plus GA_3	0.09
		plus Fe	0.16
		plus GA_3 and Fe	0.37
6	II	untreated	0.07
		plus GA_3	0.06
		plus Fe	0.32
		plus GA_3 and Fe	0.16
6	D	untreated	0.10
		plus GA_3	0.09
		plus Fe	0.39
		plus GA_3 and Fe	0.24
8	III	untreated	0.24
		plus GA_3	0.29
		plus Fe	0.61
		plus GA_3 and Fe	0.89
9	III	untreated	0.21
		plus GA_3	0.34
		plus Fe	0.45
		plus GA_3 and Fe	0.79
9	III	untreated	0.21
		plus GA_3	0.34
		plus Fe	0.45
		plus GA_3 and Fe	0.79
10	IV	untreated	0.15
		plus GA_3	0.25
		plus Fe	0.24
		plus GA_3 and Fe	0.59

samples examined. Depending on the place and time of sampling GA_3 stimulates, inhibits or has no influence on the production of biomass (Fig. 2) and on photosynthesis (Fig. 3). Results can not be correlated to the particular sampling place or to the specific time of collecting the water samples. Nevertheless all samples in which GA_3 stimulated the biomass production also exhibited stimulated photosynthesis.

At the present stage of our studies we are not able to explain the reasons for the divergent reaction of phytoplankton in various sea water samples to the action of gibberellic acid. Numerous factors can here play a role such as: type of phytoplankton population, the physiological state of these organisms, the varying pH of sea water, the presence of some inhibitors etc. Undoubtedly the changing pH of sea water might play a major role. Further studies are needed to clear up all these questions.

On the basic of the results presented we postulate that gibberellic acid stimulates the iron uptake, most probably due to the formation of assimilable GA_3-iron complex, and that this stimulation enhances the phytoplankton development which properties might reflect some phenomena occurring in natural environments.

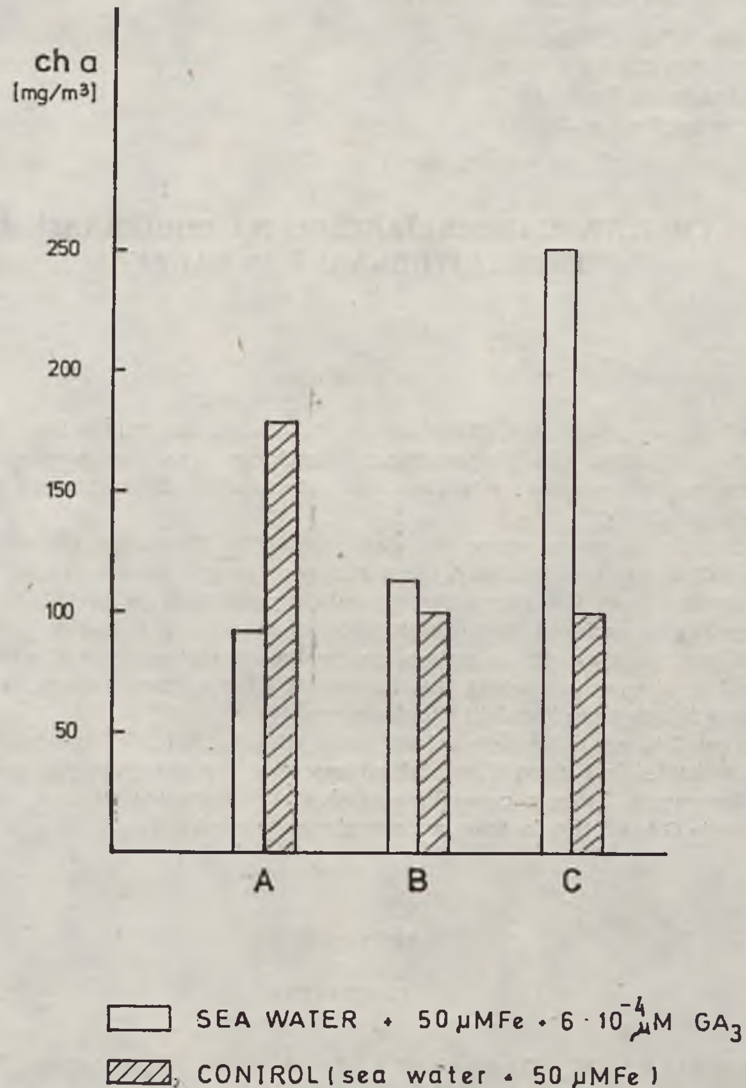

Fig. 3. Seasonal changes in the growth reaction of phytoplankton to the exogeneously applied GA_3 (the concentration of phytoplankton cells measured by the chlorophyll content)

A, B, C — different periods of testing, ch a — chlorophyll a

Ryc. 3. Zmiany sezonowe w reakcji wzrostowej fitoplanktonu na działanie egzogennego GA_3 (zagęszczenie komórek fitoplanktonu oznaczono na podstawie zawartości chlorofilu)

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WPŁYW KWASU GIBERELOWEGO NA POBIERANIE ŻELAZA PRZEZ FITOPLANKTON BAŁTYKU

Streszczenie

Zbadano wpływ kwasu giberelowego (GA_3) na pobieranie żelaza i na wielkość produkcji pierwotnej dla fitoplanktonu bałtyckiego oraz standardowych kultur fitoplanktonu inkubowanych w środowisku naturalnej wody morskiej i na podłożach syntetycznych.

Pobieranie żelaza mierzono stosując izotop ^{59}Fe. Produkcję pierwotną mierzono metodą ^{14}C, a wielkość biomasy określano poprzez pomiar zawartości chlorofilu a. Stwierdzono, że GA_3 wyraźnie stymuluje pobieranie żelaza przez fitoplankton i że zjawisko to zachodzi w ściśle określonym zakresie pH: 6—6,5. Bardzo prawdopodobne jest, że zjawisko to polega na tworzeniu się przyswajalnego kompleksu GA_3-Fe i że stymulujący efekt GA_3 na wzrost fitoplanktonu polega na zwiększeniu pobierania żelaza przez komórki fitoplanktonu.

Wpływ GA_3 na pobieranie żelaza przez fitoplankton inkubowany w naturalnej wodzie morskiej był różny i zależał od sezonu i miejsca pobrania prób. W pracy przedyskutowano, które z czynników środowiska mogą wpływać w istotny sposób na działanie GA_3 na fitoplankton w naturalnych warunkach.

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