

Effects of cobalt (Co) on marine phytoplankton species.

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ABSTRACT

The effect of cobalt (Co) on phytoplankton species was assessed both in field and laboratory experiments. Samples were taken from the Swedish West Coast (SWC) on board the R/V 'Skagerak' on July 1997; furthermore, phytoplankton cultures were grown with different Co additions in the laboratory. A cobalt detection method was developed for both total and labile Co. We used adsorptive cathodic stripping voltammetry (ACSV) by differential pulse (DP), which gave better results than linear sweep (LS), using the Co-nioxime nitrate system. Along the SWC, Co was higher at the surface, decreasing with depth. Almost all the Co found at the surface was in a labile form. Overall cobalt concentrations were higher along the SWC than in other parts of the Skagerrak Sea. The laboratory experiments showed that at least two of the phytoplankton species tested (*Synechococcus* sp. and *Chaetoceros* sp.) have a requirement for Co as a free ion, however it is not absolute. On the other hand, the haptophyte *Prymnesium parvum* showed no strict requirement for Co in its free ion form but may need vitamin B12 instead. It may also be possible that Co is competing with Zn in this species. Our data show that Co is an important micronutrient, especially in the form of a Co-cobalamine complex (B12) or any of its analogs. The question is, however, whether or not these species have the ability to produce B12 for metabolic purposes. Haptophytes comprise a group of algae that are easily found in Swedish waters, and where some of them are toxic and bloom-forming algae (i.e. *P. parvum* or *C. polylepis*). Based on our results, and assuming the species has a requirement for Co as a nutrient, a bloom of haptophytes could easily be supported in the Swedish West Coast.

Key words: Cobalt, phytoplankton, cathodic stripping voltammetry, Skagerrak Sea, Swedish West Coast, algal blooms, *Synechococcus* sp, *Chaetoceros* sp, *P. parvum*.

INTRODUCTION

The accumulation of trace metals by marine phytoplankton has received, in recent years, considerable attention because it has a direct

bearing on the nutritional and toxicological effects of metals, and on the biogeochemical cycling of metals in marine systems (Fisher and Reinfelder 1995).

Perhaps the principal characteristic of many metals in the sea is that they are essential micronutrients and must be concentrated by algal cells to meet nutritional requirements. The lack of certain metals may limit the growth of algae and thus the primary productivity in vast regions of the world's oceans. However, trace metals can also be toxic at elevated concentrations, which act in very much the same way as nutrients do; that is, in order to see their effect they need to be introduced into the cell (Fisher and Reinfelder 1995). Metal speciation in the water is perhaps the principal factor affecting metal bioavailability to marine phytoplankton; this speciation is in turn a factor of the water chemistry (Turner 1995).

When trace metals are complexed by organic particles or some other complexing agent, they become less reactive to the surface of the phytoplankton cell; the reason is that these particles and complexing agent compete with the ligands of the phytoplankton's cell membrane. Thus the presence of complexed metals and adsorption onto particles could explain the low toxicity of several trace metals present in water, even if the total concentration is high.

Trace metals can influence the growth and biomass accumulation of phytoplankton species (Harrison and Morel 1983; Murphy *et al.* 1984; Rueter and Morel 1981; Sunda *et al.* 1981; Sunda

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Received: June 1, 2011.

Accepted: September 1, 2011.

Published: November 1, 2011.

and Huntsman 1983). Such influence can be positive, by enhancing the growth and reproduction of phytoplankton; or it can be negative by limiting or inhibiting the productivity. Therefore the distribution of trace metals in the sea, and the sources and pathways they follow, may be important factors in determining the species composition in oceanic and neritic waters.

Cobalt is a trace metal that is primarily present in seawater as Co^{2+} ion, as well as its chloro-, sulfato-, and carbonate-complexes (Ahrlund 1975; Morel and Hering 1993); it can also exist as Co^{3+} organic complexes within the pH range of natural waters. Co^{2+} exists as a divalent cation, which forms labile organic complexes, while Co^{3+} forms inert complexes or oxides (Moffett and Ho 1996). Co is rapidly removed from the seawater by both biotic and abiotic mechanisms. In shallow waters, near the coast, up to 98% of the metal can be found in sediments and in suspended particulate matter (Robertson *et al.* 1973).

Cobalt is needed by the phytoplankton cell for the synthesis of cyanocobalamin (vitamin B12) and is therefore an important trace metal. This is especially true since cobalt concentrations in the open oceans are extremely low, e.g. 4-50 pM in the North Pacific (Martin and Gordon 1988; Martin *et al.* 1989) and 20 pM in the North Atlantic (Granéli and Haraldsson 1993); while they are, for example, somewhat higher in coastal areas of western Sweden, e.g. 100-1000 pM (Granéli and Haraldsson

1993). Knauer *et al.* (1982) has estimated the residence time of cobalt to be between 30 to 50 years.

Studies have shown that cobalt can enhance the growth of blue-green algae, as well as nitrogen fixation (Granéli and Risinger 1994; Holm-Hansen *et al.* 1954; Iswaran and Rao 1964). Price and Morel (1990) have shown that cobalt can substitute zinc in the metabolic processes of the phytoplankton cell. Phytoplankton is dependent on vitamin B12 for their growth (Granéli and Risinger 1994; Guillard and Cassie 1963; Ohwada and Taga 1972; Swift 1980; Swift 1981).

Based on the above, it is clear that Co is an important trace metal for phytoplankton. Our aim is to define the relation between cobalt concentrations in the water and the production levels of phytoplankton species common to Swedish waters, taking into consideration their growth rates and the way they are affected by Co.

MATERIALS AND METHODS

Study Area

The Gullmar Fjord, which lies along the Swedish West Coast, between Göteborg and Tjärnö, has been the subject of frequent oceanographic and biological observations. One characteristic that sets it apart is the fact that it's the only fjord in Sweden with a distinct sill. The fjord is 30 km long by 3 km wide and has a maximum depth of 120 m with a sill

Table 1. Positions of sampling stations occupied during the research cruise on board the R/V Skagerrak and those from the Gullmar fjord, max. depth and sample depths are included. * Only those sampling stations in which water was collected have depth information.

Station	Latitude	Longitude	Depth (m)	Sample Depths (m)*	Comments
OT1	58°11'N	11°27'E	15	5,12	Ellösefjord
OT2	58°13'N	11°32'E	14.5	5	SO de Koljöfjord
OT3	58°18'N	11°34'E	43	10,30	Kungsviken
OT4	58°16'N	11°40'E	55	5,30	Kalvöfjord
OT5	58°18'N	11°46'E	41	5,30	Havstensfjord
OT6	58°20'N	11°52'E	46	5,30	Byfjorden (Uddevala)
OT7	58°12'N	11°51'E	25	5	Ljungkile
OT8	58°06'N	11°48'E	37	5,25	Stenungsund
OT9	57°55'N	11°41'E	51	3,10,30	Marstrand (Älgön)
GF1	58°19'N	11°32'E	119	5,6,20,50,90	Djuphalan
GF2	58°16'N	11°29'E	57	7,8,20	Ingela's
GF3	58°23'N	11°37'E	61	4,5,8,20	Bjorkholmen
GF4	58°15'N	11°22'E	53	1,12	Fjord entrance

depth of 45 m (Lindahl and Hernroth 1983).

Water stratification inside the fjord is important and permanent. 3 layers normally form the water column. The first is a thin surface layer that stretches to a depth of about 20 m. This water body, which comes from the Baltic Current and thus possesses high nutrient levels, dominates the euphotic zone of the fjord and is low on salinity (18-30). Below this lies a water body of Skagerrak origin, this water's salinities ranges from 31 to 33 and normally covers from 20 to 50 m depth. Finally there is a deep-water body that lies below the sill depth, with salinities of 34-35, which comes from the North Sea and covers mostly the deep basin of the fjord (45-120 m). A somewhat pronounced thermocline inside the fjord might be found from May until September at a depth of 15 to 20 m (Lindahl and Perissinotto 1987). The Gullmar Fjord is affected by changes in wind patterns that affect the occurrence and vertical distribution of the different water masses. It's interesting to point out that water below the sill depth, that is the deep water body, is only exchanged normally during late winter or early spring when conditions permit deep circulation (Lindahl and Dahl 1988).

Field Experiments

Seawater samples were collected for analysis along the Northwest Swedish coast and within the Gullmar fjord. From July 27th to 31st a research cruise was made on board the "R/V Skagerrak", where samples from 9 stations (OT1 - OT9) were collected along the Northwest Swedish coast. In addition, water samples were also collected for analysis at 4 different stations within the Gullmar fjord, although these samples were only used for the development of the Co detection method. The position, station depth and sample depth can be found on table 2.1.

In all stations a CTD (Conductivity, Temperature and Depth) *in situ* chlorophyll profile was obtained. Samples for trace metals, along with samples for pigments and nutrients were collected using Go-Flo bottles at different depths depending on both the chlorophyll maxima and the maximum depth.

Trace Metal Sampling

The polyethylene bottles for the trace metal samples had been acid washed previously with 1M HCl, rinsed with Milli-Q water (18.2 MOhmz quality water), and rinsed with sample water twice prior to filling. Finally, sample bottles were stored in polyethylene bags and stored in a refrigerator until analysis. Throughout the sampling, special care was taken on the handling of the water to avoid any metal contamination.

All samples were filtered using air-pressure through a Nuclepore filter (pore size 0.4 μm) prior to analysis. All voltammetric experiments were carried out using a μ Autolab voltammeter (Echochemie) with a Metrohm VA 663 voltammetric cell, which was connected to a hanging mercury drop electrode (HMDE), the voltammeter and the HMDE were controlled by computer using GPES 4.4 (Echochemie) for windows.

Analysis of Cobalt

A stock 1M ammonia buffer solution was prepared by adding 16 mL of $\text{NH}_{4\text{conc}}$ to 174 mL Milli-Q water, 10 mL of HCl conc. was also added. A 5M nitrite solution, which helps enhance the cobalt signal, was prepared by adding 34.5 g of NaNO_2 to 100 mL of Milli-Q water. Finally, a 10 mM Nioxime (cyclohexane-1,2-dione dioxime) solution was used for the formation of a Co(II)-nioxime complex. For the determination of the blank, Atlantic Ocean seawater which had previously been UV-digested, was used. It was purified by passing it through a Chelex-100 column, which has an efficiency in removing cobalt of 95% (Vega and van den Berg 1997). For the determination of labile cobalt by adsorptive cathodic stripping voltammetry (ACSV), 20 mL of sample were put into the voltammetric cell, the pH was adjusted by addition of 400 μL of the pH 9.1 ammonia buffer (1M). After that, 40 μL of 10 mM nioxime and 4 mL of 5 M NaNO_2 were added. The sample was purged for 4 minutes prior to the deposition using nitrogen; this was done to remove any dissolved oxygen in the sample (Bobrowski 1990; Bobrowski and Bond 1992; Donat and Bruland 1988; Gao et al. 1996). The deposition

potential was set to -0.9 V for 60 s. After the initial measurement, subsequent additions of 40 µL of 50 nM (nanomol/kg) Co were made to enhance the signal. Cobalt concentrations were then calculated using the standard additions signal data.

Total cobalt was calculated with the same procedure as labile, except that the samples were UV-radiated to break any organic complexes. A UV system from ACE Glass was used, which has a 1200 W UV lamp. Samples were irradiated for 3 hr in acid cleaned quartz tubes.

Laboratory Experiments

Cobalt culture experiments were carried out to assess the response of phytoplankton species to different cobalt concentrations. In almost all experiments, filtered (0.4 µm pore size filter) seawater for the culture media was collected at the Kristineberg Marine Research Station, in the Gullmar Fjord, using a deep-water pump. The only exceptions were 3 experiments in which replicates were made using Ocean Scientific International filtered Atlantic seawater (General Purpose Seawater, GPS-1). In all cultures salinity was adjusted to 26 by addition of Milli-Q water.

been found to be rapid and extensive (Huntsman and Sunda 1980). Metal contamination of water samples analyzed has eliminated evidence of trace metal limitation in the medium (Brand et al. 1983). There is also a tendency of phytoplankton and bacteria to grow and accumulate on the walls of these containers that produce dense and unevenly distributed populations that don't resemble those found in nature.

The chemical changes of the added metals and the media on a time-scale are the second major issue. In most cases the addition of a chelator to a metal-enriched medium requires a period of equilibration in which a metal-chelator complex is formed, if this is not kept in mind the obtained results might be misleading and an accurate interpretation of changes in growth is impossible.

In all cases, the seawater for the media was sterilized using the microwave technique described by Keller et al. (1988). This technique is quick and doesn't contaminate the media with trace metals or cause precipitation. All nutrient and stock solutions were sterilized in the same way as the seawater.

The media for all experiments was prepared in the following way: 50 µL of 0.886 M nitrate

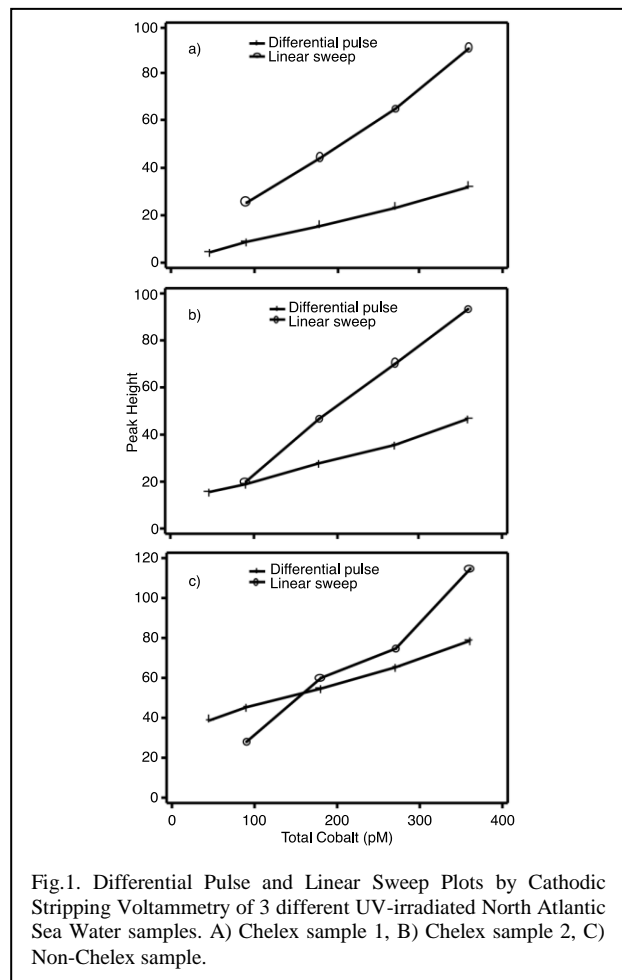
Table 2. Clone number, growth media and source of the phytoplankton species tested.

Species	Clone No.	Media	Isolator	Source
Dinoflagellates				
<i>P. micans</i> ¹	LAC 44	f/2 - Si		Lund
<i>A. carterae</i>	LAC 1	f/2 - Si	E. Graneli	Lund
Prymnesiophyta				
<i>E. huxleyi</i>	92A	f/2 - Si	W. Blackley	MarBot
<i>P. parvum</i>	LAC 39	f/2 - Si		Lund
<i>P. pouchetii</i>	LAC 31	f/2 - Si	L-Å. Gisselson	Lund
<i>C. polylepis</i>	LAC 52	f/2 - Si	L. Elder	Lund
Cyanobacteria				
<i>Synechococcus sp.</i>	DC2	f/2 - Si	L. Brand	MarBot
Diatoms				
<i>Chaetoceros sp.</i>	LAC 19	f/2 + Si	L-Å. Gisselson	Lund

Several issues need to be discussed when growing phytoplankton cultures from natural populations using natural water, so as to avoid any contamination in the growth medium and to obtain results as close as possible to those found in nature. The first of these issues is the use of glass and plastic containers for the cultures. Adsorption of several metals onto glass and plastic surfaces has

(NO₃⁻), 0.0746 M phosphate (PO₄³⁻) and 10 mM iron (FeIII) as a citrate complex each were added as nutrients; furthermore, 50 µL of 0.089 M silicate were added for the diatoms. 50 µL of stock f/2 vitamin solution were added only to control cultures. 100 µL of 50 mM (0.05M) EDTA were added to each culture as complexing agent, for a final concentration of 100 µM EDTA. Finally,

cobalt additions were made by adding 10 μL and 100 μL of 50 μM cobalt standards for a final



concentration of 10 nM and 100 nM respectively, and leaving one culture without cobalt. All cultures were done with 4 replicates of each Co concentration (0, 10 and 100 nM), while only one experiment had 3 replicates.

All cultures were inoculated with 1 mL of stock phytoplankton cultures for each species tested. Those which were grown at the Analytical and Marine Chemistry (AMK) department were kept under constant illumination at around 22 °C, while the Marine Botany (MB) cultures were maintained at 12 °C under cyclic illumination (11 hL:13 hD) provided by white fluorescent lamps.

Control culture species (Table 2) included 4 Prymnesiophyta (*Emiliana huxleyi*, *Prymnesium parvum*, *Phaeocystis pouchetii* and *Chrysochromulina polylepis*), 1 diatom (*Chaetoceros* sp.), 2 dinoflagellates (*Prorocentrum micans* and *Amphidinium carterae*) and 1 blue-green algae (*Synechococcus* sp.). From the first set of experiments (AMK), chlorophyll a fluorescence was measured daily using a fluorometer. These cultures included *P. micans*, *A. carterae*, *C. polylepis* and *Synechococcus* sp.

Of the MB cultures only 4 species responded to the culture media and were used for the cobalt culture experiments. Three of them (*P. parvum*, *P. pouchetii* and *E. huxleyi*) were counted daily for 5 days with a FACSCalibur Flow Cytometer (Becton and Dickinson), while *Synechococcus* sp. was counted manually every second day for 10 days using a light microscope. Cell concentrations per liter were calculated and plotted.

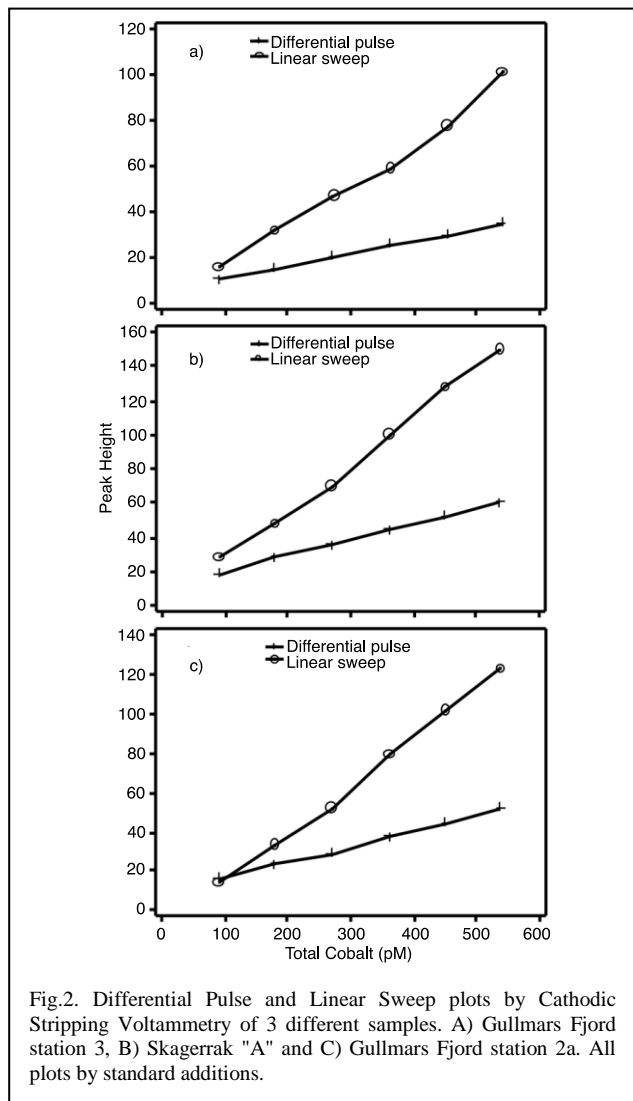
RESULTS

Development of CoII-detection Method

The first stage was comprised by the development of a Co-detection method that would suit the needs of the samples to be tested. Adsorptive cathodic stripping voltammetry (ACSV) was used for the determination of both total and labile cobalt.

The blank measurement was found to be 46 pM (picomol/kg) (± 12 pM 3SD) and the analytical precision at 100 pM level was approximately 6%.

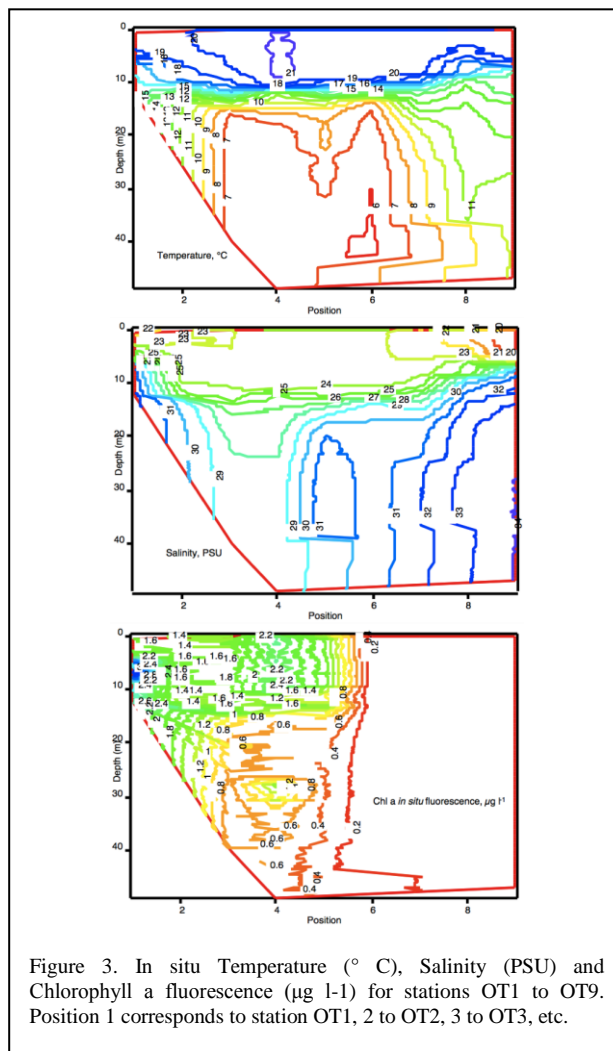
Standard additions of Co to UV-irradiated Seawater from the North Atlantic are shown on Fig.1, these samples had been passed through a chelex column and then analyzed using DP and LS. In all cases we found that the sensitivity [$\text{nA nM}^{-1} \text{min}^{-1}$] was higher with LS than with DP, however the signal to noise ratio was better with DP. For all Co additions, DP gave a linear response, while LS showed non-linearities at high Co concentrations with a shift in the peak potential, making peak height analysis more complicated.



An example of standard additions experiments on non UV-irradiated water samples using DP and LS can be seen on Fig.2. In this case 3 different samples (2 from the Gullmars fjord: GF3 collected on 970613 and station GF2a collected on 970709; and one from the Skagerrak sea collected on 961002) were analyzed using DP and LS. These samples behaved much in the same way as the samples in Fig. 3. While the sensitivity was also higher with LS than with DP, DP gave better linear responses than did LS.

Field Experiments

The total and labile cobalt concentrations for the samples collected along the Swedish west coast (OT samples) from Orust to Tjörn are collected in Table 3. Included are also the



labile/total Co ratios for the same OT samples. The lowest total cobalt concentrations were found at station OT8 (Stenungsund) at 25 meters (ca. 150 pM), while the lowest labile cobalt was found at station OT5 (Havstens fjord) at 30 meters depth (ca. 30 pM). *In situ* temperature, salinity and Chl a for stations OT1 to OT9 are plotted on Fig.3. Finally, total cobalt (pM) obtained at 4 different stations in the Gullmar fjord, at two different dates (Jul 4th, Aug 29th 1997) is shown in Fig.4. Overall, the data

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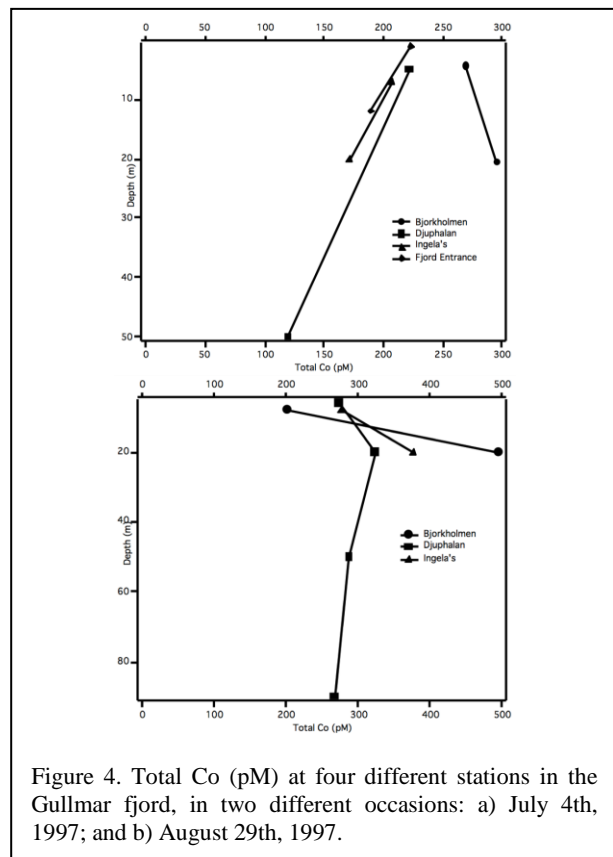
Linares, F. y Croot, P.

Received: June 1, 2011.

Accepted: September 1, 2011.

Published: November 1, 2011.

show that in the fjord Co was higher further inland, towards Bjorkholmen.



Laboratory Experiments

Results from the laboratory culture experiments are gathered in Fig.5. Relative growth rates (μ), which are doublings per day calculated from the Log of the in situ Chl *a*, were plotted for each phytoplankton species against the estimated free cobalt concentration (pCo). Distinction was made between EDTA and DTPA complexing agents, since they seemed to affect the results differently, because of the observed differences in the free metal concentrations for the other metals involved, particularly Zn (see Tables 4 a,b).

DISCUSSION

Development of Co (II)-detection Method

It is a known fact that recent improvements in

sampling techniques and analytical methods for the measurement of trace metals in natural waters have allowed, to a great extent, major advances in the understanding of trace metal chemistry in the sea (Jickells and Burton 1988).

In a first set of experiments the catalytic effect of nitrite was tested. According to Bobrowski and Bond (1992), the sensitivity of a cobalt complex (i.e. Co-DMG, Co-Nioxime) is improved about 20 fold by the catalytic effect of nitrite; our own tests showed such an increase in the Co signal and all subsequent experiments contained nitrite in them (Fig. 1 and 2).

While we were developing our Co-detection method, a paper by Vega and van der Berg (1997) came to our attention. The method described by Vega and van den Berg was similar to the methodology we had developed, based on the work of Bobrowski and Bond (1992), for the direct determination of picomolar levels of cobalt by ACSV using a cobalt-nioxime complex with nitrite catalysis. We followed the procedure of Vega and van den Berg, throughout the rest of our experiments, with no major modifications to their protocol.

One important question that developed while measuring the trace metal was: what cobalt species was being measured by the ACSV method? According to Byrne *et al.* (1988), Co^{2+} is only weakly complexed in inorganic seawater and at pH 8.2 as much as 74% of the metal is found as a free hydrated ion. On the other hand, according to Zhang *et al.* (1990), Co (II) can form strong organic complexes in seawater. However, in the deposition step of the DPCSV method, most Co^{3+} organic complexes are reduced to Co^{2+} (e.g. Costa *et al.* (1971) found that Co^{3+} -DMG complexes appeared to undergo an overall irreversible reduction to Co^{2+}), and hence the analyte measured by the voltammeter is thought to be Co^{2+} .

ACSV measures free metal ions and metals that are easily dissociated from complexes or colloidal particles. This method does not, by itself,

measure the total cobalt content but it measures the amount of analyte in a labile form. This labile cobalt may be viewed as analogous to the biologically available form of the metal, which would include the free ion form as well as species that are easily dissociated in aqueous solution. Certain metal complexes are bound to organic ligands such that dissociation cannot take place in the time scale of the measurement, and therefore the metal is not detected. For the measurement of total cobalt, UV-irradiation of the sample destroys these organic Co complexes, rendering inorganic cobalt available for direct measurement by ACSV.

Initially, we examined the sensitivity for cobalt using the Vega and van der Berg protocol, both under differential pulse (DP) and linear sweep (LS) conditions (Figures 3.1 and 3.2). Vega and van der Berg had found DP to give the best results with nioxime, however Bobrowski (1990) reported better sensitivity using LS for the Co-nioxime nitrite system.

Interestingly, when comparing the results of figure 3.2 with the UV irradiated samples (Figure 3.1); the sensitivity was only slightly reduced, and indicating a small effect from natural surfactant material that may compete for space on the mercury drop. We observed linear responses to cobalt additions from DP and significant Co signals at the zero addition level, this may indicate the following: (1) strong organic Co complexation of the residual non labile Co in the sample, (2) the existence of a nioxime labile Co pool present in the unmodified sample, and (3) rapid complexation of the added Co by nioxime, suggesting little or no competition from natural Co complexes.

Based on the experiments previously described, we concluded that DP would be the best method for the determination of labile and total cobalt. This is in contrast to what Bobrowski and Bond (1992) suggest, where they found that LS produced a better resolution and showed advantages related to speed; however in their experiments they used DMG (Dimethylglyoxime) as a complexing agent, while we decided to use Nioxime (cyclohexane-1,2-dione dioxime) which, according to Donat and Bruland (1988), has a high selectivity for Co similar to DMG and forms complexes with Co (II) enhancing the

adsorption into the HMDE. They also found that Co sensitivity in the presence of Nioxime was 2.5 to 20 times greater for ammonia buffered samples than in the presence of DMG.

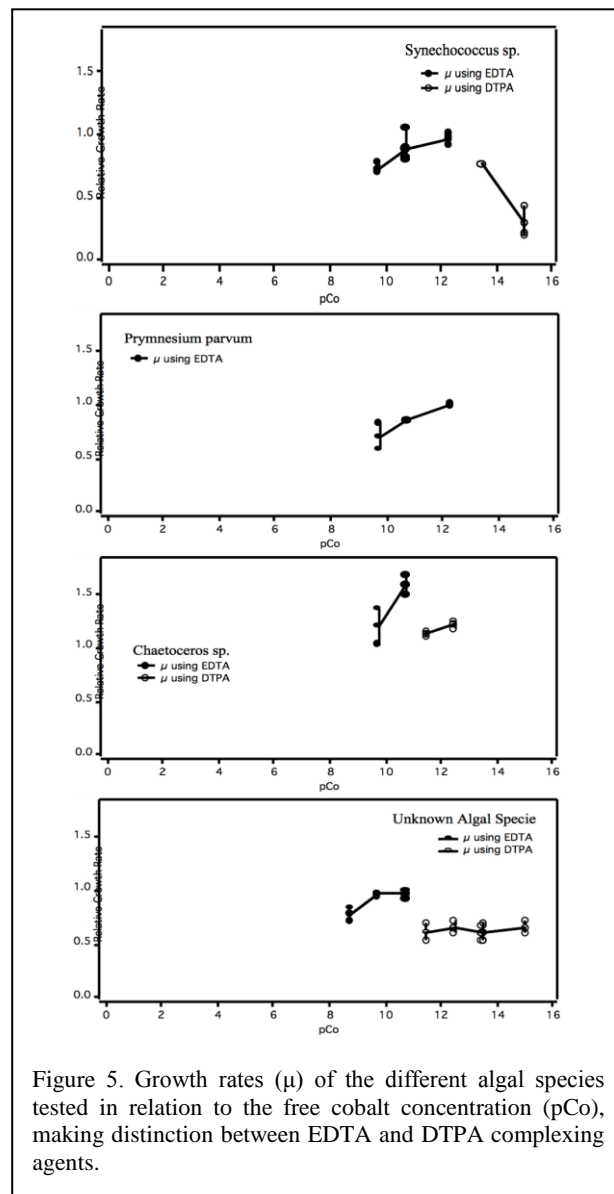


Figure 5. Growth rates (μ) of the different algal species tested in relation to the free cobalt concentration (pCo), making distinction between EDTA and DTPA complexing agents.

Finally, nioxime forms stronger complexes with Co (II), early work by Zhang *et al.* (1990) had shown that the detection window of DMG was weaker than natural complexes.

Field Experiments

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Accepted: September 1, 2011.

Received: June 1, 2011.

Published: November 1, 2011.

In the OT transect (Table 1), both the highest total and labile cobalt concentrations were found at the surface in station OT4 (near the Kalvö fjord). However, the values at this station are unusually large (ca. 1200 pM for total and labile cobalt), compared to the other stations, which may indicate that the samples were contaminated prior to the measurement. It is also possible however that there was industrial run off in the area. According to the Report on the chemistry of seawater IV by the Dept. of Analytical and Marine Chemistry GU/CTH (1967) (AMK 1967), very low O₂ levels were found in the Uddevalla fjord system, particularly at Koljo (58°13.65N 11°34.40E) and Byfjorden (58°20.00N 11°52.65E). They further suggest that there may be high metal levels in Havstensfjord (58°18.60N 11°45.20E) probably caused by industries near Stengunsund. These samples had a suboxic smell and further sampling showed that other trace metals such as Cu and Zn were found to be also high in this sample (Croot, pers. comm.).

From the data collected at the OT sites, it is

clear that cobalt was higher at the surface slowly decreasing with depth (Table 1), According to Granéli and Haraldsson (1993), in coastal waters the concentrations of trace metals can be appreciably higher than in offshore waters due to the addition of metals from river water. Moreover, it is estimated that two thirds of the cobalt flux to the sea is derived from rivers (Chester 1990).

What is even more interesting is that cobalt was found to be, in general, higher in the Swedish West Coast than in the Skagerrak (see Croot *et al.* 2002). The influence of the Baltic Current is clearly seen in the Swedish West Coast. To this effect, North Atlantic water analyzed by Granéli and Haraldsson (1993) contained around 20 pM of total Co, while concentrations were somewhat higher for the Baltic and Kattegat.

According to Table 1 the highest non-labile cobalt for the OT stations was also found near the surface in stations OT3 and OT4, which were the ones that showed the highest total and labile cobalt values. The labile/total cobalt ratio (Table 1)

Table 3. Total and labile Co for the OT stations. Non-labile cobalt and the Labile/Total Co ratio are also shown.

Station	Depth (m)	Labile [Co] pM	Total [Co] pM	Non Labile [Co] pM	Lab/Tot [Co]
OT1	5	167	224	57	0.75
OT1	13	94			
OT2	5	105	262	157	0.4
OT3	10	159	392	232	0.41
OT3	30	699	174		4.01a
OT4	5	1250	1161		1.08b
OT4	30	454	586	132	0.78
OT5	5	267	513	246	0.52
OT5	30	28	181	153	0.16
OT6	5	308	358	50	0.86
OT6	30	405	384		1.05b
OT7	5	258	335	76	0.77
OT8	5	121	202	80	0.6
OT8	25	82	149	67	0.55
OT9	3	144	184	40	0.78
OT9	10	150	185	35	0.81
OT9	30	62	164	102	0.38

a. The sample was probably contaminated.

b. Within error (3 std. dev.) its 100% of total almost all is labile.

showed that at least 50% of the cobalt found at the surface is in labile form, with values increasing up to 80-85% in stations OT6 and OT7 (Byfjorden near Uddevalla). When compared to the in situ fluorescence measurements (Figure 3.4), the algae showed a tendency to concentrate in the mixed layer and congregate just above the pycnocline, where

towards the fjord entrance; the importance of this is that given the case, an algal bloom that would require Co as a nutrient might start from the inside of the fjord and move towards the entrance and to open sea. Further studies are needed however to understand these relationships better.

Table 4a. Free metal ion composition of media (EDTA).

Metal	Total [M] nM	$\log([M]_{\text{tot}}/[M]_{\text{f}})$	$-\log([M]_{\text{f}})$
Co	0.3	2.72	12.24
Cu	6	5.21	13.43
Cd	0.3	2.87	12.39
Fe	500	11.51	17.81
Mn	100	0.28	7.28
Zn	10	2.91	10.91

[M]_{tot}/[M]_f ratio calculated for each metal under the following conditions: 10 μM EDTA in KMF seawater (S = 26), $\log K^{\circ}\text{Co(II)EDTA} = 7.72$, $\log K^{\circ}\text{CuEDTA} = 10.21$, $\log K^{\circ}\text{CdEDTA} = 7.87$, $\log K^{\circ}\text{Fe(III)EDTA} = 7.72$, $\log K^{\circ}\text{MnEDTA} = 5.28$ and $\log K^{\circ}\text{ZnEDTA} = 7.91$.

Table 4b. Free metal ion composition of media (DTPA).

Metal	Total [M] nM	$\log([M]_{\text{tot}}/[M]_{\text{f}})$	$-\log([M]_{\text{f}})$
Co	0.3	5.51	15.03
Cu	6	7.79	16.01
Cd	0.3	5.44	14.96
Fe	500	14.24	20.54
Mn	100	1.84	8.84
Zn	10	4.64	12.64

[M]_{tot}/[M]_f ratio calculated for each metal under the following conditions: 10 μM DTPA in KMF seawater (S = 26), $\log K^{\circ}\text{Co(II)DTPA} = 10.51$, $\log K^{\circ}\text{CuDTPA} = 12.79$, $\log K^{\circ}\text{CdDTPA} = 10.44$, $\log K^{\circ}\text{Fe(III)DTPA} = 19.24$, $\log K^{\circ}\text{MnDTPA} = 6.84$ and $\log K^{\circ}\text{ZnDTPA} = 9.64$.

most of the labile cobalt was found.

Individual pigment information for the OT stations is not available, and because of that reason no conclusion can be drawn as to what algal groups were predominant in that area at that point of time. Finally, although there was no pigment information for the Gullmar fjord stations either; what is interesting to note is that cobalt was found to be, overall, higher in Bjorkholmen, which lies further inland. Cobalt concentrations tended to decrease

Laboratory experiments.

Synechococcus sp. appeared to be affected both by DTPA and by low concentrations of Co (Figure 3.5), while the algae grew fairly well with EDTA as complexing agent and at high concentrations of Co. Granéli and Risinger (1994) found that the growth rates of the alga *Chrysochromulina polylepis* were not affected by Co additions that had EDTA as a complexing agent. However it should be noted that the experiments that contain EDTA were grown at the Department of Marine Botany (MB), while the DTPA experiments were grown at the Department of Analytical and Marine Chemistry (AMK). The differences in the growing conditions may have also affected the growth rates of the samples, since we noticed that some species never grew because of the higher temperature or lack of some trace nutrient when they were at the AMK department.

The biomass and productivity of picoplankton species in the sea (i.e. *Synechococcus* sp.), is such that these species play a significant role in the cycling of metals in marine ecosystems; furthermore, these algal species are seasonally important in the Skagerrak during summer (Karlson and Nilsson 1991). Fisher (1985) found that accumulation of metal by *Synechococcus* sp. was rapid, with equilibrium established after 24 hr. Moreover, *Synechococcus* sp. cells accumulated metal in proportion to the dissolved metal concentration found in the media. In our experiments, *Synechococcus* sp. was among the species that grew well despite the lack of nutrient enrichment (i.e. B₁₂) and the observed rates seem to indicate that *Synechococcus* sp. may not need B₁₂ to grow, but also that it has low Co requirements for growth. To this effect, Sunda and Huntsman (1995) found that despite the small cell diameter of *Synechococcus* sp. (ca. 0.7 μm), which increases diffusion of trace metals through the cell because of

its high surface: volume ratio, Co uptake rates were a third to a tenth of rates found in other algal species.

The fact that in our experiments *Synechococcus* sp. appeared to be unaffected by high Co levels (Fig. 5) indicate that its requirement is not absolute, but it exists however. Sunda and Huntsman (1995) found this same trend on *Synechococcus bacillaris* and further found that the algae seemed to need Co but not Zn to grow. To this effect, in one of our early experiments, we found a negative effect on the growth of *Synechococcus* sp. when Zn was added to the culture with DTPA.

Growth rates in *Prymnesium parvum* (Fig. 5) decreased as the Co levels increased, this may indicate that the algae has no strict requirement for Co^{2+} but may require B_{12} instead, Co may also be competing with Zn at high concentrations, thus displaying the given result. However, the only complexing agent used was EDTA and thus no comparison can be made to other complexing agents. Muggli and Harrison (1996) found an effect of EDTA on algal cells at high EDTA concentration. Some studies have shown that when Co is added as B_{12} , methionine production improves in *Prymnesium parvum* (Swift 1980). *Prymnesium parvum* is a Haptophyta that has been reported to cause toxic blooms (van den Hoek *et al.* 1995). Swift (1980) argues that among the haptophytes there is a requirement only for thiamin in the majority of the species, however several of them also require Co as B_{12} in conjunction with thiamin.

Chaetoceros sp. (Figure 5) showed an increase in growth rate with EDTA, but no apparent effect at low concentrations of Co with DTPA. The differences in these growth rates were significant nonetheless when comparing these two chelators. Both sets of experiments had the same conditions and these differences are either attributed to the chelators themselves, or that the algae were negatively affected by Co additions. Zuleika Segatto (1996) found that the biomass of the marine diatom *Skeletonema costatum* was indeed affected by Co additions. Another possible explication for may be that the great majority of diatoms require Co as B_{12} to grow (Swift 1980), while only a small percentage have no requirement at all.

Finally, unidentified green algae (Fig. 5) with two flagella ($380 \mu\text{m}^3$ in volume, roughly spherical with $4.5 \mu\text{m}^3$ in radius, isolated from KMF in March 1997) were grown under different pCo levels and with two different chelators (EDTA and DTPA). While the algae grew fairly well under all cobalt concentrations, there were some minor differences between the complexing agents used. DTPA appeared to have little effect on the growth of the alga, while EDTA allowed for better growth rates. It was not possible for us to identify the algae but based on the results it seems that the species has no requirement for Co or B_{12} , since there were no major differences between the control and the additions. There are a few species which have absolutely no requirement for Co at all (Swift 1980), however, without a positive identification of the algae in question, no conclusion can be drawn as to whether the algae indeed had no requirement for Co or not.

Differences between Co^{2+} and B_{12} Requirements in Phytoplankton.

The phytoplankton species tested in this study comprise a wide range of algal groups and they all appeared to be affected differently by Co. The blue-green algae *Synechococcus* sp. showed a requirement for Co^{2+} , but it was not absolute. On the other hand the haptophyte *P. parvum* displayed no strict requirement for Co^{2+} but didn't seem to be affected by low concentrations of Co. Studies have shown that some of these species need B_{12} to grow (Swift 1980). Granéli and Risinger (1994) in a culture experiment of the haptophyte *Chrysochromulina polylepis* with Co and B_{12} , found that chlorophyll accumulation ceased after 8 or 9 days for all treatments except the ones that had vitamin B_{12} as a Co source. Finally the diatom *Chaetoceros* sp. had no strict requirement for Co, but was stimulated by it; this is may be explained by the fact that this species has a requirement for B_{12} . Swift (1981) found that, at least in the Gulf of Maine, centric diatoms from the spring bloom lacked an absolute requirement for B_{12} but were in fact stimulated by it.

As it has been stated elsewhere (Ohwada and Taga 1972; Swift 1980), it is possible that the

differences observed in the concentrations between vitamin B₁₂ and those of other vitamins such as thiamin and biotin in phytoplankton species, could suggest that thiamin and biotin are produced and excreted into the media by dominant species of the phytoplankton (thus following closely the trend of chlorophyll a), while vitamin B₁₂ would be taken up by these same species during these same periods (i.e. auxotrophic diatoms). Confirm thus the importance of B₁₂ and indeed Co²⁺ as a nutrient.

There are differences in the way in which organisms accumulate Co. In natural waters it appears to come directly from the diet, whereas in laboratory experiments it's accumulated mostly from water. Studies have shown that besides taking up vitamin B₁₂ from the medium, some phytoplankton species can also secrete a binding-protein, which renders the vitamin unavailable to other organisms (Droop 1968). In fact the replacement of a ligand by another molecule (be it another ligand or a solvent molecule) is one of the fundamental reactions of metal ions in solutions and often an important feature of biological systems (Costa et al. 1971).

CONCLUSIONS

The cobalt detection method proved to be effective for the measurement of both total and labile Co. Furthermore; differential pulse was found to give better results than linear sweep using the Co-nioxime nitrate system.

Almost all the Co found at the surface was in a labile form. Along the Swedish West Coast, Co was higher at the surface, which most of it was also in labile form, decreasing with depth.

High cobalt concentrations (total and labile) can be attributed to either local runoff coming from the coast, or to the origin of the water, which seems to come from the Baltic.

Based on the laboratory experiments, it can be concluded that at least two of the phytoplankton species tested (the blue-green algae *Synechococcus* sp. and diatom *Chaetoceros* sp.) have a requirement for Co as a free ion, but it is not absolute. The haptophyte *P. parvum* showed no strict requirement for the free ion form of Co but may need B₁₂

instead. It may also be possible that Co is competing with Zn.

Our data show that Co is an important micronutrient especially in the form of a Co-cobalamine complex (B₁₂), the question is whether or not these species have the ability to produce B₁₂ for metabolic purposes. Haptophytes comprise a group of algae which are easily found in Swedish waters, and where some of them are toxic and bloom-forming algae (i.e. *P. parvum*). Based on our results, and assuming the species has a requirement for Co as a nutrient, a bloom of haptophytes could be supported in either the Skagerrak or the Swedish West Coast.

ACKNOWLEDGMENTS

The work carried could not have been possible without the assistance of the following people: Dr. Greg Morrison, Dr. David Turner, Dr. Margareta Wedborg and Dr. Bengt Karlson for their valuable comments on the manuscript. The staff at the AMK department, as well as and the staff at the Marine Botany Department for providing a place to work. To the R/V 'Skagerak' crew; the 'Arne Tiselius' and the Kristineberg Marine Station for help with the logistic work. Financial support for this work was provided by a scholarship from the Swedish Foundation for International Cooperation in Research and Higher Education (STINT).

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