

Metalloporphyrins in recent sediments of the Baltic Sea*

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Metalloporphyrins
Nickel(II)
Vanadyl(II)
Sediments
Sea
Baltic

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Abstract

Recent sediments of the southern Baltic Sea were investigated for the possible occurrence of metalloporphyrins. The content of both vanadyl(II) and nickel(II) metalloporphyrins estimated in the sediment samples studied is of the order of magnitude $(1-6) \times 10^{-2} \mu\text{g g}^{-1}$ (d. w.). The results also suggest that the porphyrins in recent Baltic sediments are of anthropogenic origin. The highly significant correlation of the metalloporphyrin content with chlorophyll *a* indicates either that these metalloporphyrins are formed in the water column or that phytoplankton contribute to metalloporphyrin transfer from water to sediments.

1. Introduction

Metalloporphyrins, namely nickel(II) and vanadyl(II) complexes, are compounds widespread in different aqueous sedimentary environments (Quirke, 1987). Nevertheless, our present knowledge of their geochemistry is incomplete in many respects and requires further investigation. In particular, the geochemistry of porphyrins in the marine environment is very poorly known.

It is usually assumed that metal chelation with nickel(II) and vanadyl(II) ions is the last step in chlorophyll diagenesis according to the Treibs scheme (Filby and van Berkel, 1987). However, it is unclear where the metal insertion occurs – in the water column or in the sediments. According to the

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literature, the latter possibility seems more probable as metalloporphyrins are found in deep sediment layers (about 200 m sub-bottom) (Baker and Louda, 1982; Palmer and Baker, 1978).

The Baltic is a sea of shallow, brackish waters and slow exchange of water with the North Sea. These characteristics, together with the increasing eutrophication of the basin, make it an exceptional model for studies of contemporary tetrapyrrole geochemistry. The latest work (Kowalewska, 1994) has indicated that chlorins, *i.e.* chlorophyll *a* and the products of its early diagenesis, make up the main tetrapyrrole fraction in the recent Baltic sediments. In the present work the sediments were investigated for the possible occurrence of metalloporphyrins.

2. Material and methods

2.1. Samples

Table 1. Characteristics of the sampling stations
(t, S and O₂ in the bottom waters)

| Station | Depth [m] | Temperature [°C] | Salinity [psu] | *O ₂ [% stn] |
|--------------------|--------------|---------------------|-------------------|----------------------------|
| Puck Bay (92A) | 35 | 8.7 | 7.4 | 60 |
| Gdańsk Deep (G-2) | 108 | 5.3 | 10.4 | 1.7 |
| Bornholm Deep (P5) | 92 | 6.0 | 15.6 | 1.3 |

*Kowalewska, 1994.

Sediment samples were collected during a cruise of r/v 'Oceania' in the southern Baltic Sea in October 1992. The samples were collected with a Niemistö-type core sampler. The location and characteristics of the sampling stations are presented in Tab. 1 and Fig. 1. After collection, the samples were divided into layers from 1 to 5 cm thick, transferred to polyethylene containers and frozen at -20° on board. The analyses were carried out in a shore laboratory.

2.2. Preparation of metalloporphyrin standards from petroleum

Two types of crude oil were used for the preparation of the metalloporphyrin standards: 1. Soviet blend, one of the kinds of petroleum used by the Gdańsk refinery, and 2. petroleum originating from sources under the bottom of the southern Baltic Sea. The method used to isolate the metalloporphyrins was in part similar to the procedure described by Hajibrahim *et al.* (1978). First, the petroleum sample, diluted with toluene, was extracted with methanol several times and the methanol layers separated

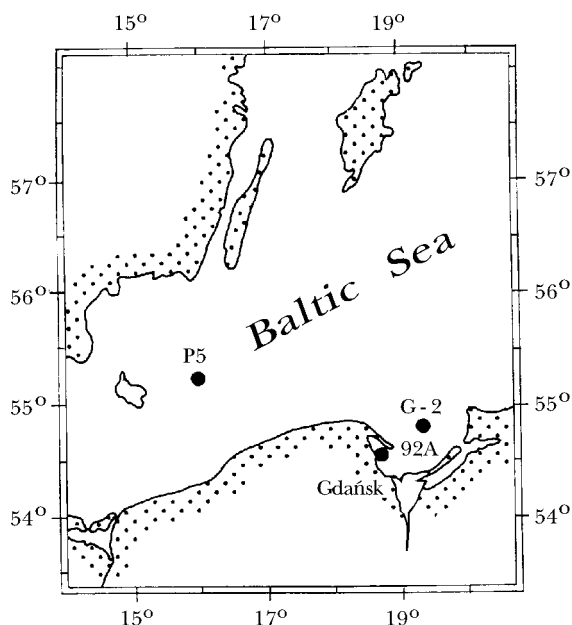


Fig. 1. Location of the sampling stations in the southern Baltic Sea, October 1992

and collected. Next, the solvent was evaporated and the residue dissolved in toluene. Metalloporphyrins were separated by column chromatography (Silica gel 60, Machery Nagel Co., Germany). Hexane, hexane:toluene 1:1 (v/v) and toluene:chloroform 1:1 (v/v) were used successively for elution of the column. The collected porphyrin fractions in hexane:toluene and toluene:chloroform were evaporated to dryness and further purified by TLC (Merck Kieselgel 60, acetone:hexane 20:35 v/v). The metalloporphyrin concentration in the sample was determined by the spectrophotometric method, assuming the mean extinction coefficient of metalloporphyrins at the Soret band to be 2×10^5 and at the major non-Soret band to be 2×10^4 (Hodgson and Baker, 1967). Sample purity was checked by HPLC (Figs. 2, 3). Analysis of the absorption spectra of these fractions indicated that the petroleum samples contained mainly vanadyl porphyrins (Hodgson and Baker, 1967; Palmer and Baker, 1978).

2.3. Isolation of metalloporphyrins from sediments

A frozen sample (5.0–100 g) was allowed to thaw, then centrifuged to remove water. The sediment was next extracted several times with acetone:methanol (9:1) (Palmer and Baker, 1978) using sonication. After each extraction the sample was centrifuged and the supernatant collected and reextracted with benzene in the system acetone+methanol:benzene:water

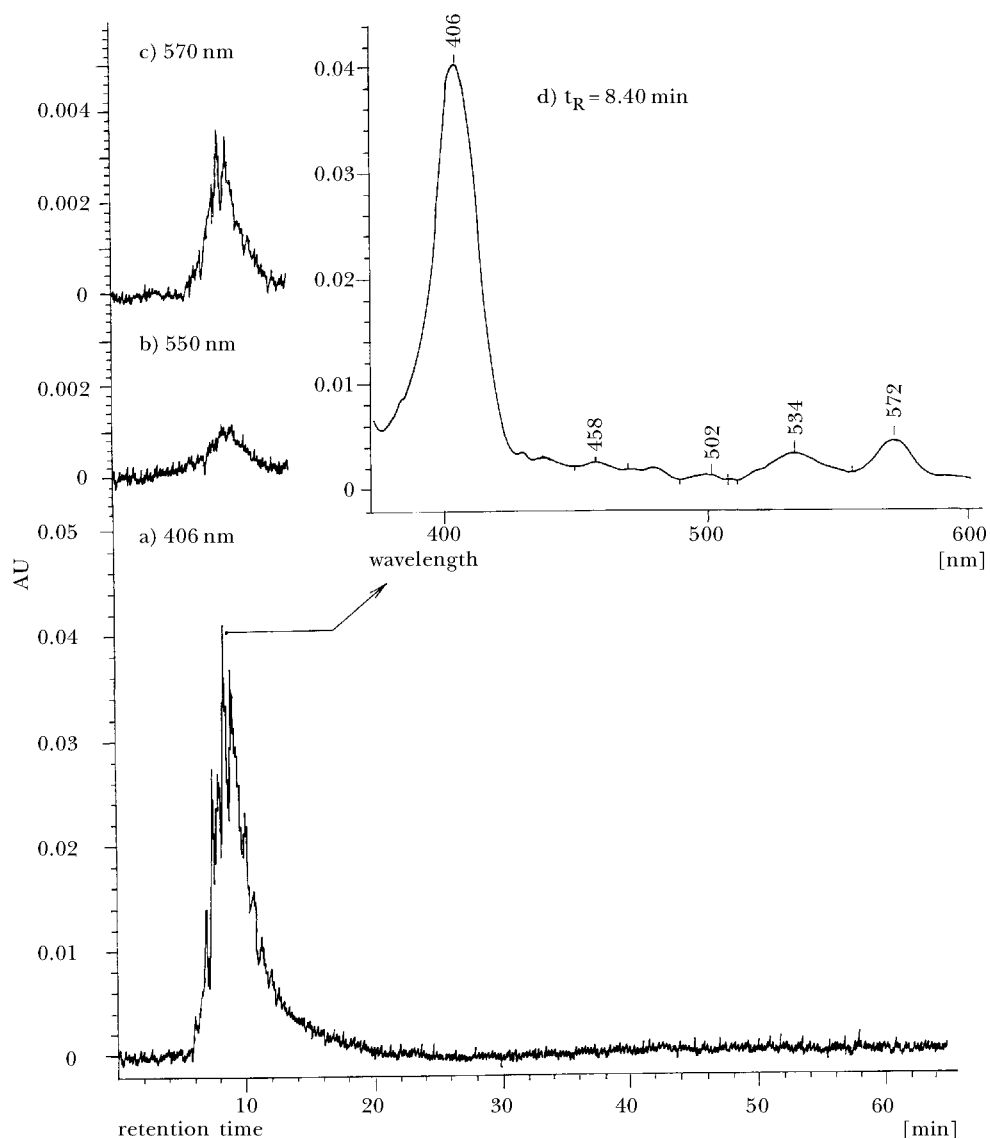


Fig. 2. Metalloporphyrin standard isolated from petroleum (Soviet blend): HPLC chromatograms at a) 406 nm, b) 550 nm, c) 570 nm; d) spectrum at retention time $t_R = 8.40$ min

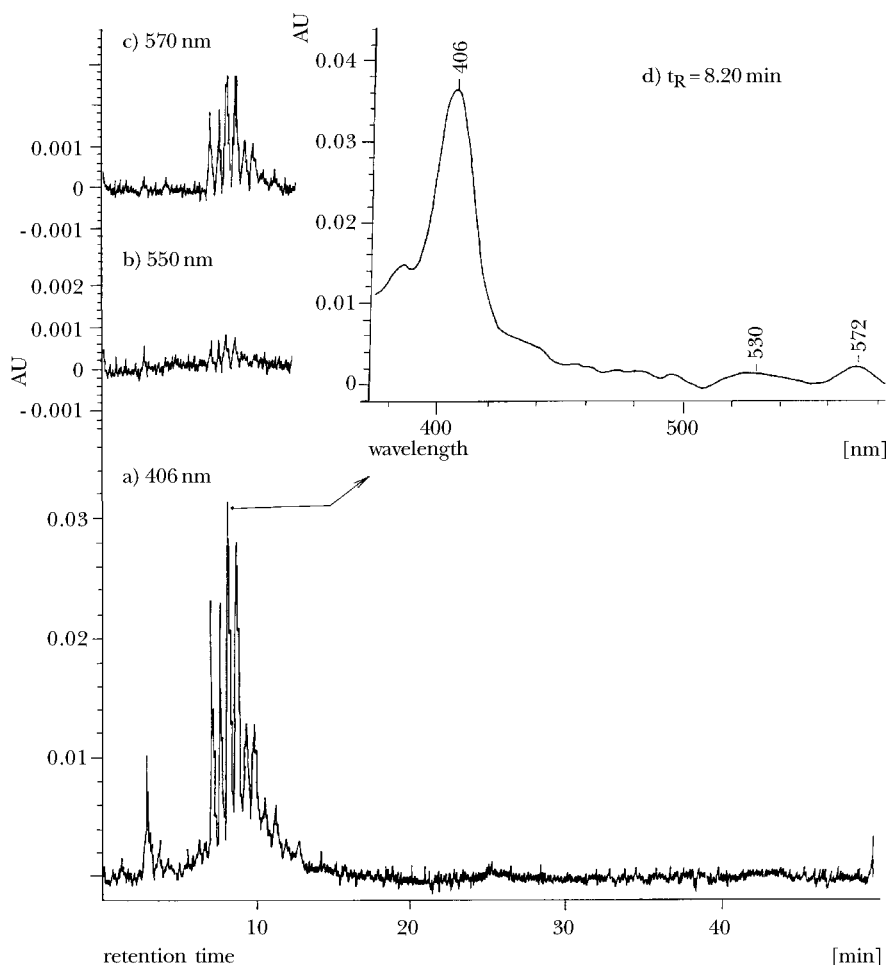


Fig. 3. Metalloporphyrin standard isolated from petroleum (Baltic Sea sources): HPLC chromatograms at a) 406 nm, b) 550 nm, c) 570 nm; d) spectrum at retention time $t_R = 8.20$ min

50:5:45 (v/v/v). The benzene layer was separated and evaporated to dryness under vacuum. The residue was dissolved in a few millilitres of toluene and applied to a silica column (Silica gel 60, Machery Nagel Co., Germany, bed volume 4.0 cm \times 2.7 cm i.d.). The column was subsequently eluted with hexane (to remove hydrocarbons, elution controlled by fluorescence in UV), hexane:toluene 1:1(v/v), toluene:chloroform 1:1 (v/v) and acetone, as in the isolation of metalloporphyrins from petroleum. The fractions eluted with hexane:toluene and toluene:chloroform (of red colour, no fluorescence in UV) were evaporated to dryness, dissolved in a small volume of acetone and analysed by HPLC.

2.4. High-performance liquid chromatography

The separations were carried out according to a modified procedure for chlorins (Kowalewska, 1993, 1994). The extract dissolved in acetone (20 μ l) was injected into a chromatograph system (Knauer, Germany) equipped with a diode-array type detector (Chrom-a-Scope). Separations were carried out on a reversed-phase column with the chemically bonded phase (Merck Lichrospher 100 RP 18 end-capped column, 250 \times 4 mm, 5 μ m with a Merck Lichrospher 100 RP 18 end-capped precolumn 4 \times 4 mm, 5 μ m). The mobile phase was acetone-water in two gradient systems: I – 0 min, 80:20; 5 min, 85:15; 20 min, 95:5; 40 min, 95:5; 50–65 min, 100% acetone; 70 min, 80:20; flow rate 1 ml min⁻¹ or II – 0 min, 80:20; 10 min, 85:15; 20 min, 95:5; 40–55 min, 100% acetone; 65 min, 80:20, flow rate 0.5 ml min⁻¹. Reproducibility in two subsequent injections was about 5% at porphyrin concentration 0.5 μ g ml⁻¹.

2.5. Calculation of metalloporphyrin content

As one can see from the spectra registered by the diode-array detector at a retention time corresponding to the highest peak of metalloporphyrin standards isolated from petroleum (t_R = 8.5 min and 17–19 min, depending on the gradient used), a mixture of nickel and vanadium metalloporphyrins was coeluted. A carotenoid was also eluted there, although its absorption at 406 nm was assumed to be negligible compared to that of the metalloporphyrins. The lack of red fluorescence in TLC proved that free bases were absent from this fraction. The metalloporphyrin content was estimated on the basis of the ratio of the metalloporphyrin peak area (t_R = 8.5 min or 17–19 min) to the total area of all the chromatogram peaks and absorption of the sample at 406 nm measured by spectrophotometer (Shimadzu, type UV-1202).

3. Results and discussion

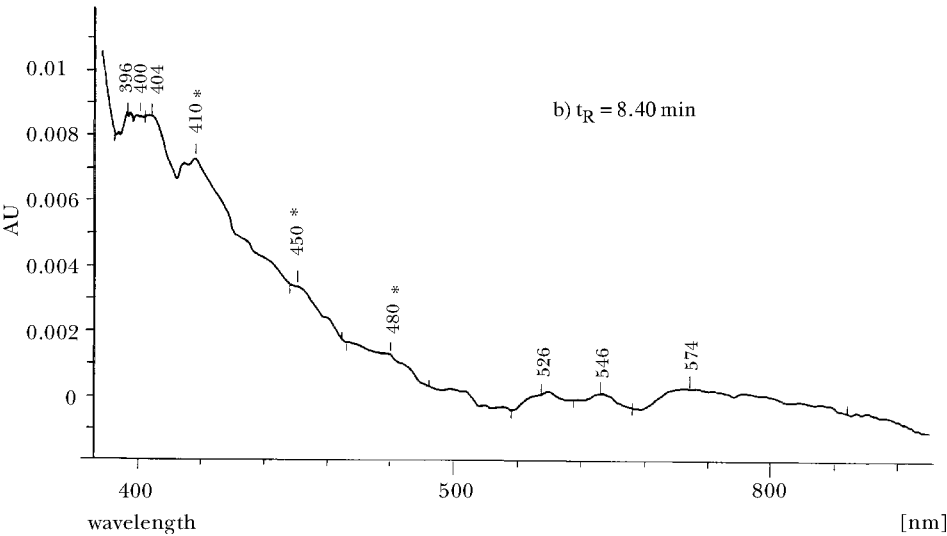
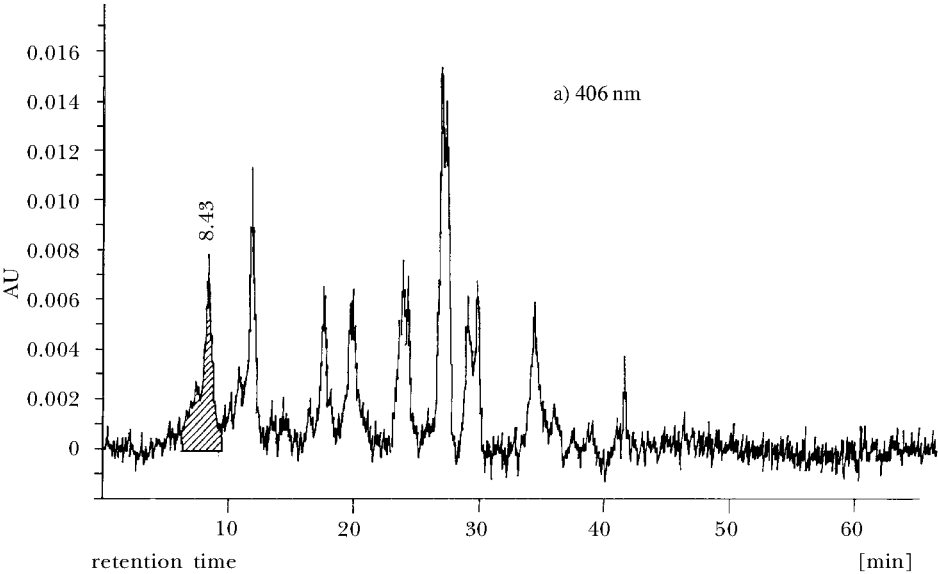
The sampling stations for collecting material were chosen in such a way that they covered the very wide variety of conditions existing in the Polish Baltic economic zone. These were two deepest areas – the Gdańsk Deep (G-2) and the Bornholm Deep (P5), where the halocline occurs. The former is a sink, into which local currents deposit particulate matter transported to the sea by the River Vistula and flocculated by contact of freshwater with seawater. In the Bornholm Deep, which is situated in the open sea, a better water exchange with the North Sea occurs than in the Gdańsk Deep. Puck Bay (92A), the inner part of the Gulf of Gdańsk, is a eutrophic region of shallow waters, without a permanent halocline.

Examples of HPLC chromatograms of metalloporphyrins isolated from sediments and partially purified by column chromatography are shown in Fig. 4. The spectra recorded indicate that both vanadyl and nickel porphyrins occurred in the sediment extracts. The metalloporphyrin content estimated for the sediments studied is of the order of magnitude $(1-6) \times 10^{-2} \mu\text{g g}^{-1}$ of dry sediment (Tab. 2), which is low compared to the literature data for marine shales ($0.001-120 \mu\text{g g}^{-1}$) (Palmer and Baker, 1978), but considerable in comparison with the metalloporphyrin content in petroleum originating from sources under the bottom of the Baltic (about $700 \mu\text{g l}^{-1}$).

Table 2. Metalloporphyrin content in the Baltic Sea sediments studied

| Station | Layer [cm] | Metalloporphyrins [$\mu\text{g g}^{-1}$ d. w.] |
|--------------------|---------------|--|
| Puck Bay (92A) | 0 – 1 | 0.025 |
| | 1 – 5 | 0.005 |
| Gdańsk Deep (G-2) | 0 – 1 | 0.058 |
| | 1 – 5 | 0.064 |
| | 5 – 10 | 0.049 |
| Bornholm Deep (P5) | 0 – 5 | 0.015 |
| | 5 – 10 | 0.012 |

The highest metalloporphyrin content was found in the sediments from the Gdańsk Deep. Such a result suggests that metalloporphyrins in recent Baltic sediments are of anthropogenic origin and may be markers of petroleum seepages. Simultaneously, however, the correlation coefficient of the metalloporphyrin content with that of chlorophyll *a*, determined for the same sediment samples, is significant ($r = 0.8$ at $p < 0.02$). For phaeophorbide *a* – a marker of zooplankton grazing – the correlation is distinctly lower ($r = 0.58$ at $p < 0.2$). Such a high correlation with chlorophyll *a* implies



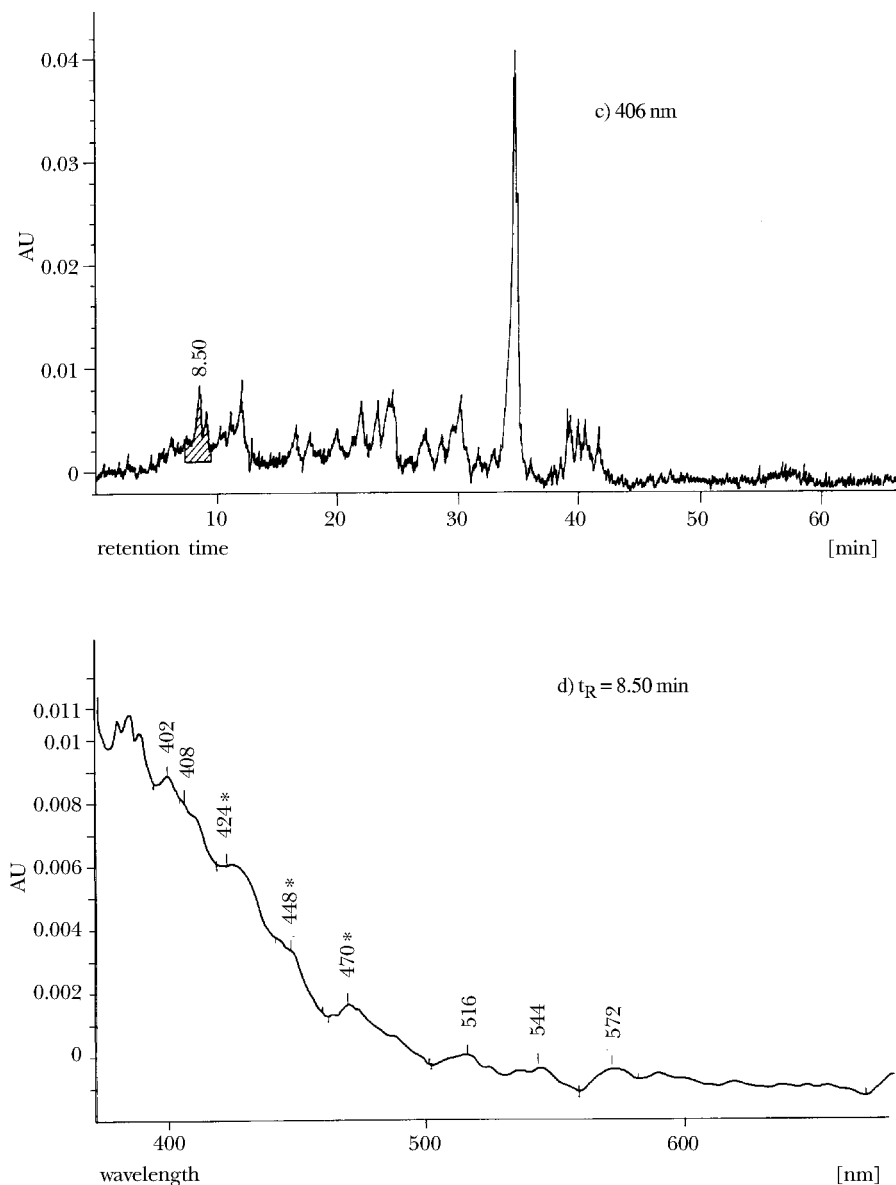


Fig. 4. Sediment from Puck Bay (station 92A, 0–1 cm sediment layer): fraction eluted from silica gel column in hexane:toluene (1:1, v/v): a) HPLC chromatogram at 406 nm, b) spectrum at retention time $t_R = 8.40$ min; fraction eluted from silica gel column in toluene:chloroform (1:1, v/v): c) HPLC chromatogram at 406 nm; d) spectrum at retention time $t_R = 8.50$ min; * asterisks denote maxima of a coeluting carotenoid

either that metalloporphyrins are formed in the water column or that phytoplankton make a contribution to metalloporphyrin transport from the water column to sediments.

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