Determination of indole-3-acetic acid in sediments of the southern Baltic Sea*

OCEANOLOGIA, 40 (2), 1998.
pp. 117–124.

KEYWORDS
Auxin
Indole-3-acetic acid (IAA)
Marine sediments
Southern Baltic

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Manuscript received March 27, 1998, in final form May 13, 1998.

Abstract

Analyses of indole-3-acetic acid (IAA) in sediments collected at stations in the southern Baltic Sea were carried out by HPLC. The seasonal variations in IAA content as well as the relationship between organic matter content and the concentration of IAA were shown. A decrease in IAA concentration with depth was observed in the sediment profiles from the Gdańsk Deep, the Bornholm Deep and Słupsk Furrow.

1. Introduction

Despite the availability of substantial data on the origin, quantity and distribution of organic matter in the sea, our knowledge of its individual components has been scarce for a long time. This was mainly because of their presence in trace amounts and the lack of specific and sensitive methods of determining them. With improvements in analytical procedures for the concentration, extraction and analysis of organic compounds, the elemental composition of organic matter in the sea has gradually come to be known. Some of the substances biosynthesised, metabolised and secreted by marine organisms or released from bottom sediments display biological activity. Moreover, there is an increasing body of evidence showing that

* Presented at the 3rd Conference 'Chemistry, Geochemistry and Protection of the Marine Environment', Sopot, 12th December 1997.

traces of growth regulators, vitamins, toxins and other biologically active compounds in the sea exert a considerable influence on algal communities in that they affect the growth, morphogenesis and reproduction of these organisms (Bonin *et al.*, 1981).

Indole-3-acetic acid (IAA) is a ubiquitous plant hormone essential for normal growth and is involved in almost every developmental process during the whole life cycle of the plant. Like plant hormones in general, IAA is present in plant tissues at very low concentrations (10–100 ng g^{-1} fresh mass) (Olsson *et al.*, 1996). However, not only plants but also numerous microorganisms are known to be capable of synthesising IAA. IAA-like substances have been found in some species of green, brown and red algae, which are regarded as a less advanced group of organisms than the higher plants (Provasoli and Carlucci, 1974; Augier, 1976; Bradley, 1991; Evans and Trewavas, 1991). Bentley (1960), Maruyama *et al.* (1989), and Krevs and Jankevicius (1992) recorded the presence of auxin-like substances in seawater and marine sediments. In these last two papers, marine bacteria, including those living in bottom sediments, are considered a potential source of IAA in the sea.

Mass spectrometry coupled with gas chromatography provided unequivocal identification of IAA in northern Adriatic waters (Mazur and Homme, 1993) and in the marine sediments from the Gulf of Gdańsk (Mazur *et al.*, 1997). Research into the temporal and spatial distribution of IAA in the Gulf of Gdańsk carried out by HPLC showed that the IAA content in this environment was subject to seasonal variation (Mazur, 1994). The latest studies have been focused on measuring the IAA content in different layers of marine sediments collected at particular stations in the southern Baltic Sea.

2. Materials and methods

In February, May, July and October 1994 samples of superficial sediments (0–5 cm) were collected with a corer from stations A (30 m), B (35 m) and C (7 m) in the Gulf of Gdańsk (Fig. 1). The sediments collected at the last-mentioned station were sandy, while the others were black and muddy. In September 1995 sediment cores were collected from the Gdańsk Deep (114 m) and the Bornholm Deep (93 m), and in May 1996 from the Gdańsk Deep and Słupsk Furrow (92 m). On board ship the cores were divided into 2 cm sections and stored at –20°C. These sediments were black, muddy and smelt of hydrogen sulphide, which is indicative of highly anaerobic conditions. The individual sediment sections (*ca* 70 g each) were suspended in 1 dm^3 of distilled water. After continuous stirring for 8 h the samples were centrifuged (at 4.000 *g* for 20 min) and the liquid phase was removed. The residue was twice resuspended

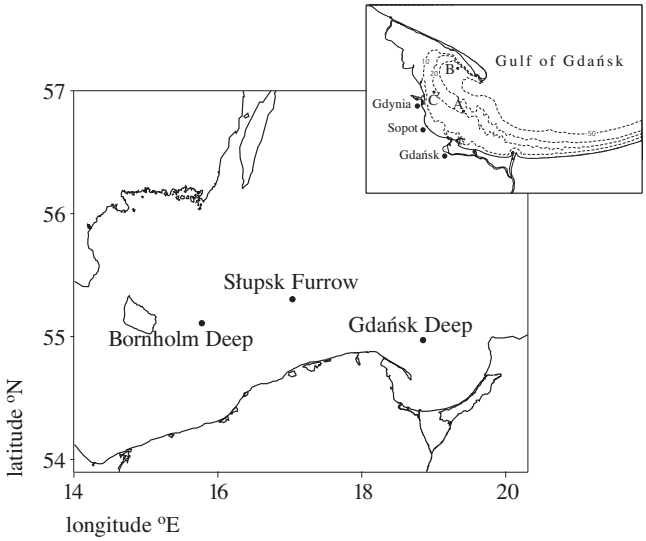

Fig. 1. Location of sampling stations

in 0.5 dm^3 distilled water, stirred for 1 h and centrifuged again. The water extracts from the sediments were combined, then adjusted to pH 3 with 6 M HCl, filtered through a Whatman GF/F glass microfibre filter and passed through an Amberlite XAD–4 column (4.5×1.4 cm I.D.) at a flow rate of 3 cm^3 min^{-1} (Fig. 2). The resin was washed with 25 cm^3 distilled water and the sorbed substances then eluted with 25 cm^3 of methanol.

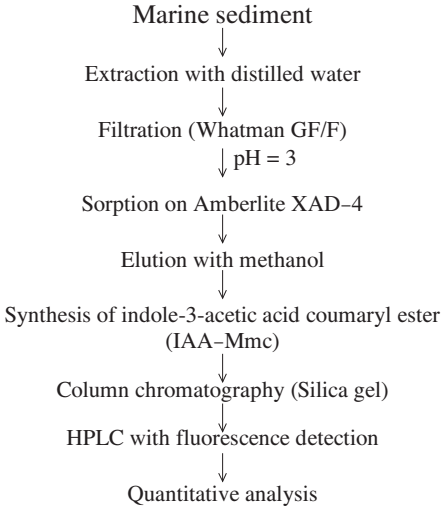

Fig. 2. Procedure for the determination of indole-3-acetic acid (IAA) in marine sediments

The alcohol fraction from XAD–4 was evaporated to dryness *in vacuo* at 30°C. The residue was dissolved in 0.5 cm^3 of acetonitrile and derivatised with a 10 μmol dm^{-3} solution of 18-crown-6 and a 1 μmol dm^{-3} solution of 4-(bromomethyl)-7-methoxycoumarin (BrMmc) (Ertel and Carstensen, 1987; Mazur *et al.*, 1997). After the reaction, the mixture was cleaned up by passage through a silica gel column (3.5×1 cm I.D.). To remove interfering substances, such as the excess and decomposed products of the reagent, the column was washed with 25 cm^3 of *n*-hexane-ethyl acetate (5:1, v/v). The main fraction was eluted with 25 cm^3 of *n*-hexane-ethyl acetate (3:1, v/v), evaporated *in vacuo* at 35°C, redissolved in 1.0 cm^3 of acetonitrile and analysed in a Waters chromatographic system. The injection volume was 20 μl and separations were performed on a 100 RP–8 Lichrospher column (250×4 mm I.D., 5 μm packing). A linear gradient from 50 to 98% of methanol was run in 30 min, at a flow rate of 0.5 cm^3 min^{-1} and ambient temperature. Fluorimetric detection was performed at an excitation wavelength of 312 nm, the emission being monitored at 399 nm.

3. Results and discussion

HPLC analyses of the derivatised organic substances extracted from marine sediments showed the presence of a compound with a fluorescence emission spectrum and retention time identical to that of authentic IAA coumaryl ester. The identity of the peak was confirmed by co-chromatography with standard IAA–Mmc. The IAA content in sediment samples was calculated from the peak areas using a chromatogram integrator. The presence of IAA in marine sediments had been proved before with a GC–MS method (Mazur *et al.*, 1997).

The analyses of superficial sediments collected at stations A, B and C in the Gulf of Gdańsk revealed the highest IAA content in the May samples collected at organic-matter-rich stations A (6.3 nmol kg^{-1}) and B (2.5 nmol kg^{-1}) (Fig. 3). A considerable concentration of IAA was also recorded in the samples taken at these stations in July (3.9 nmol kg^{-1} and 2.0 nmol kg^{-1} respectively). In February and October the IAA content in the superficial sediments at stations A and B turned out to be much lower and fell within the range of 0.23–0.30 nmol kg^{-1}. In the sediments collected from station C, IAA was not detected at concentrations higher than the detection limit of the method (20 fmol of IAA) at any season of the year. Lack of IAA in the sandy sediments collected at station C and the detection of IAA in the muddy sediments from stations A and B are indicative of the relationship between the organic matter content in the samples analysed and the presence of IAA.

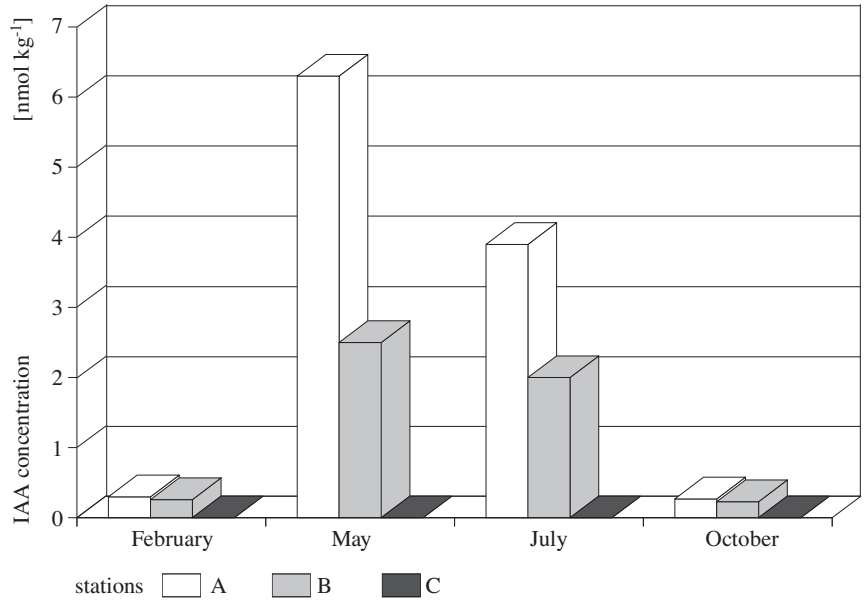

Fig. 3. IAA concentration in surface sediments collected from stations A, B and C in the Gulf of Gdańsk

The analyses of IAA in the sediment cores taken from the Gdańsk and Bornholm Deeps, and from Słupsk Furrow showed that in successive sections of the cores its content decreased with depth. Only the upper two sections of the sediments collected in September 1995 were analysed. In the Gdańsk Deep the IAA content in the 0–2 cm section of the sediment was 33.7 nmol kg^{-1}, in the 2–4 cm section it was 25.6 nmol kg^{-1} (Fig. 4). In the Bornholm Deep the IAA content in the 0–2 cm section of the core was 36.8 nmol kg^{-1}, in the 2–4 cm section it was 28.5 nmol kg^{-1}. The analyses of the sediments collected in May 1996 showed that IAA was present down to the 6–8 cm section of the sediment cores from the Gdańsk Deep and Słupsk Furrow (Fig. 5). The IAA contents in different sections of the sediment core collected from the Gdańsk Deep were as follows: 0–2 cm – 27.1 nmol kg^{-1}; 2–4 cm – 26.1 nmol kg^{-1}; 4–6 cm – 11.6 nmol kg^{-1}; 6–8 cm – 98 pmol kg^{-1}. IAA was not detected in the 8–10 cm section of the core. In the case of the sediment core collected from Słupsk Furrow, the IAA contents in different sections were as follows: 0–2 cm – 32.1 nmol kg^{-1}; 2–4 cm – 27.2 nmol kg^{-1}; 4–6 cm – 7.1 nmol kg^{-1}; 6–8 cm – 61 pmol kg^{-1}. IAA was not detected in the 8–10 cm section of this core. It was noticed that the IAA content in the 0–2 cm sections was the highest, while in the 2–4 cm sections it was from 67 to 96% lower. However, a pronounced fall in IAA content was found in the 4–6 cm and 6–8 cm

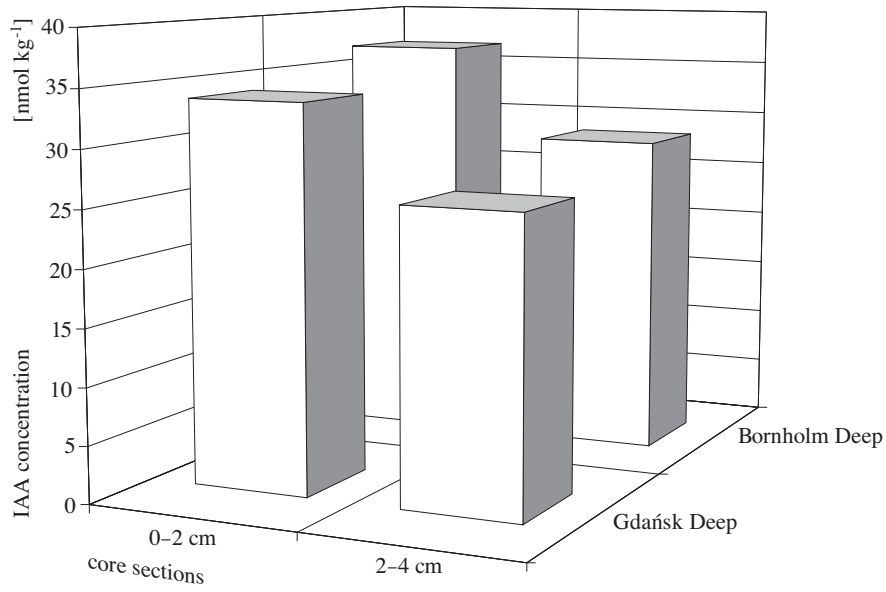

Fig. 4. IAA concentration in two sections of the sediment cores collected from the Gdańsk and Bornholm Deeps in September 1995

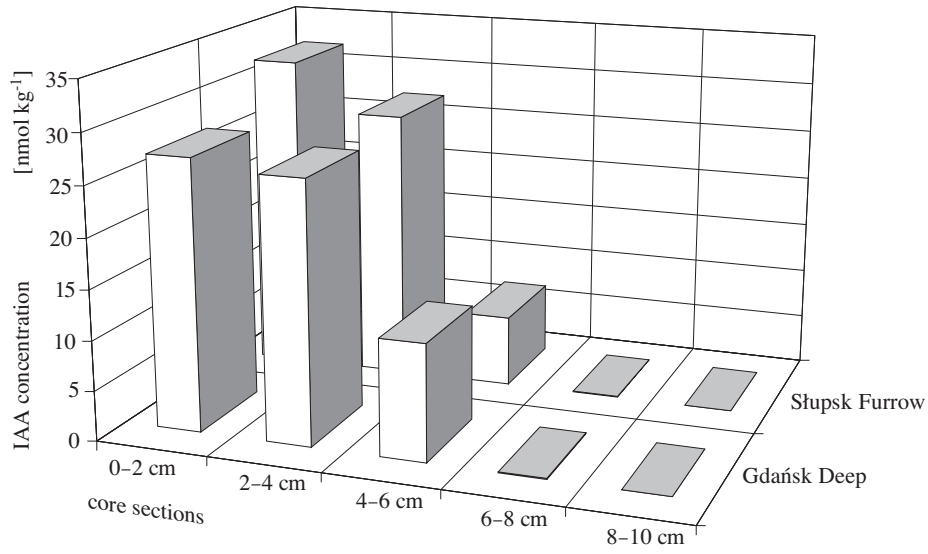

Fig. 5. IAA concentration in different sections of the sediment cores collected from the Gdańsk Deep and Słupsk Furrow in May 1996

sections of the core. Comparison of the superficial sediments collected at stations A and B of the Gulf of Gdańsk with the 0–2 cm sections of the cores from the Gdańsk and Bornholm Deep and Słupsk Furrow shows that

the IAA content was higher in the latter samples. These differences in IAA content corresponded to the organic matter content in the sediment samples analysed.

The source of IAA in the marine environment has not been defined as yet. The studies carried out by Bentley (1960) indicated that the presence of IAA in seawater was connected with the metabolic activity of planktonic organisms. Maruyama *et al.* (1989) stated that some strains of bacteria living in the marine sediments are able to produce IAA. Moreover, this process was significantly intensified in the presence of tryptophan, which among terrestrial plants and bacteria is known to be a common precursor of IAA. Originating from this central auxin precursor, different pathways of auxin biosynthesis have been described (Kawaguchi and Syono, 1996). The microbial conversion of tryptophan to IAA probably occurs via indole-3-pyruvic acid. In the marine environment, it is presumably the microbial decay of the organic matter that has settled on the sea bed following the phytoplankton blooms and the subsequent conversion of tryptophan to IAA as a general function of bacterial communities, that constitutes the source of IAA. However, in order to prove this hypothesis, the sequence of degradation reactions in sediments from the starting material through intermediate products to IAA as the final degradation product would have to be examined.

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