Polyunsaturated aldehydes as bioactive secondary metabolites in the aquatic environment

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Abstract

Polyunsaturated aldehydes (PUAs) are compounds that can be released into the natural aquatic environment by phytoplankton as bioactive secondary metabolites. These metabolites aid photosynthetic organisms in important functions as protection, competition, and species interactions. The production of PUAs result from the lipoxygenase-mediated degradation of free polyunsaturated fatty acids released by phospho- and galactolipids hydrolysis. This reaction is activated immediately after the cell integrity is broken by lysis or mastication by grazers. PUAs have negative impact on algae grazers causing carcinogenic and mutagenic effects. The aim of the presented studies is to identify polyunsaturated aldehydes in natural freshwaters. The highest PUAs concentrations were identified in autumn and in waters with high concentration of organic matters. A high coefficient of correlation (r = 0.93) was determined between the total concentration of aldehydes identified in natural surface waters and the absorbance measured at a wavelength of 254 nm characteristic for aromatic compounds. The influence of other factors such as temperature, sun-light, and presence of natural bacteria on the destruction of aldehydes was also estimated. A technique of gas chromatography with the electron capture detector and derivatization process with o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine was applied for qualitative and quantitative determination of PUAs.

Keywords: Polyunsaturated aldehydes, secondary metabolites, freshwaters, gas chromatography

1. Introduction

One of the most popular organic compounds identified in the environment are aldehydes. This ubiquitous compounds can be found in aqueous and in terrestrial environment as well as in the troposphere (Dabrowska and Nawrocki, 2013; Dabrowska *et al*. 2014). They can originate from a number of sources like oxidation processes, photochemical transformations, life processes of animals and plants, and decomposition of organic matter. One group of this compounds are polyunsaturated aldehydes (PUAs). They are volatile compounds commonly released into the environment by different phytoplankton species. The main producers of this fatty-acid-derived secondary metabolites are diatoms which constitute about 40 % of the ocean primary production (Bartual and Ortega, 2013; Leflaive and Ten-Hage, 2009, Vardi *et al*., 2006; Wolfram *et al*., 2015). Their ecological and evolutionary success in marine environment has been attributed to such factors as: strong mechanical defenses, high growth rates, ability to efficiently utilize available nutrients and capacity of certain diatoms to produce toxic compound (Taylor *et al*., 2009). This unicellular algae that live suspended in the water column can undergo periods of rapid growth, called blooms. When algal blooms die, organic compounds including polyunsaturated aldehydes are released to the surrounding water. Nanomolar concentrations of these aldehydes (mainly octadienal) can persist in the environment after the collapse of the bloom (Bartual and Ortega, 2013). These compounds can be formed also in the response to environmental stresses like wounding, grazing by predators, or competition for limiting nutrients (Ribalet *et al*., 2014). The production and release of PUAs result from the lipoxygenase-mediated degradation of free polyunsaturated fatty acids released by phospho- and galactolipids hydrolysis. This reaction is activated immediately after the cell integrity is broken by lysis or mastication by grazers (Michalec *et al*, 2013; Ribalet *et al*., 2008; Wichard *et al*., 2005; Vidoudez and Pohnert, 2008; Vidoudez *et al*., 2011). These compounds play an important role in the interactions between different species of plankton. There are mediators in chemical defense and communications (Vidoudez *et al*., 2011). PUAs interfere with the reproductive success and recruitment of diatom predators, therefore bloom can persist despite sustained grazing pressure (Michalec *et al*., 2013). Polyunsaturated aldehydes and their precursors have a negative impact on a various range of organisms such as crustaceans, annelids, bacteria, algae and yeast (Taylor *et al*., 2009). They are responsible for reduction in egg production and hatching success, production of abnormal teratogenic nauplii showing poor post-embryonic development and high mortality in copepod species which are diatoms predators (Brugnano *et al*., 2016; Ianora and Miralto, 2010; Ribalet *et al*., 2008, Vardi *et al*., 2006). According to the Vidoudez *et al*. (2011) approximately a third of marine diatoms are capable of producing PUAs so these compounds can be frequently identified in the oceanic waters where diatoms form intense blooms. The key role in the release of polyunsaturated aldehydes play the following species: *Skeletonema marinoi*, *Skeletonema costatum*, *Thalassiosira rotula*, *Phaeocystis pouchetii* (Brugnano *et al*., 2016; Hansen and Eilertsen, 2007; Michalec *et al*., 2013; Taylor *et al*., 2009). On the other hand, the type and quantity of released PUAs depend on the strain, culture age, and nutrient availability. 2E,4E/Z-octadienal and 2E,4E/Z-decadienal are the most common PUAs present in the environment (Bartual and Ortega, 2013). The purpose of the presented studies was:

a) to identify whether PUAs are produced in freshwaters together with aliphatic aldehydes

b) to estimate the average concentration of octadienal and decadienal in monitored water samples.

2. Method Description

2.1. Sample collection

Water samples were collected systematically during the years 2015-2016 from urban area of Poland in Morasko (north part of Poznan) and from the rural area in Zielonka Forest Region. The samples were taken from the top of the lake − layer of epilimnion (a depth of 40-50 cm). Since aldehydes are easily biodegradable, the aqueous samples were derivatized on the day of their collection or the samples were protected from the biodegradation by addition of biocide (copper sulphate of 95% purity from CHEMPUR).

2.2. Analysis of aliphatic and polyunsaturated aldehydes

High polarity and reactivity of aldehydes in aqueous matrices imposes the need for their derivatization, since derivatives are less polar, more volatile, and can be detected using selective detectors. The use of derivatization process has allowed to reach a lower detection limit of aldehydes. Aldehyde analysis was based on the derivatization reaction with PFBOA (O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) as a derivatizing agend to obtain oximes that, after extraction with organic solvent, were analysed by gas chromatography (GC 8000 series, Fisons Instruments) with ^{63}Ni electron capture detection. The Rtx-5MS (Restek) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film) was applied for separation. Helium and nitrogen were used as a carrier gas and a detector make-up gas, respectively. The standards of C1-C10 alifatic aldehydes and octadienal, and decadienal were purchased from Aldrich, PFBOA of > 98 % purity and hexane of > 95 % purity were from FLUCA and BAKER. DataApex Clarity™ was used for collecting and processing of chromatographic data. The evaluation of data and statistical analysis was performed by the Division of Signal Processing and Electronic Systems.

2.3. Spectroscopic analysis

Absorbance of water samples was measured by M501 Single Beam Scanning UV/Visible Spectrophotometer (Camspec) at 254 nm wavelength and cuvette with fused body with optically polished quartz windows was applied. The reference sample was ultra-pure water produced in laboratory by Millipore Synergy UV Water Purification System.

3. Result and discussion

Bartual and Ortega (2013) showed that octadienal and decadienal are the most popular compounds in marin waters among other polyunsaturated aldehydes prodused by phytoplankton, thus these two characteristic PUAs were monitored by us in freshwater samples collected from artificial reservoir, located on the campus of Adam Mickiewicz University in Morasko and from natural pond located in rural area in Boduszewo. The map with the places of sampling is presented in Figure 1 and the structure of the monitored PUAs is demonstrated in Figure 2. Both studied reservoirs have a similar area of approximetly 800 m^2 and their surface is well sun-drenched, both reservoirs are abundant in phytoplankton during vegetation season. Organoleptic observations showed high influence of organic matter on water color. Chlorophyll *a* is a common indicator used for the assessment of phytoplankton quantities in water. Chlorophyll *a* is present in the plants, algae, and photosynthesizing bacteria (e.g. blu-green algae). Chlorofill concentrations in the samples collected during the vegetation period are the highest, corresponding to the highest biomass of phytoplankton. Physico-chemical characteristic of waters is presented in Table 1. A close correlation exists between dissolved organic carbon (DOC) concentration and UV absorbance at a wavenlength of 254 nm in natural waters. The average value of absorbance was significally higher in water samples from natural pond located in rural area (0.338) than in samples collected from reservoir in the University area (0.058). A significant diference between values of turbidity measured in NTU (nephelometric turbidity unit), was also observed. Average turbidity in the samples from Boduszewo was on the level of 33.4 NTU, while in the samples from Morasko the value of turbidity amounted only 1.6 NTU. Alifatic aldehydes belong to a group of compounds commonly found in the environment. Aldehydes were identified in the lake waters and formaldehyde, acetaldehyde and propanal were the most important carbonyl compounds (Dabrowska, 2013). The seasonal presence of aldehydes can be explained as a result of the vegetation processes. C1-C10 alifatic aldehydes were found by us in the monitored waters. The total average concentration of C1-C10 aliphatic aldehydes in samples from Morasko was on the level of 17.1 μg/l, with the maximum value of 43.5 μg/l. The total average concentration of C1-C10 in samples from Boduszewo was higher and amounted 30,6 μg/l with the maximum of 64.2 μg/l. The range of PUAs concentration for the monitored waters is shown in Figure 3. Generally, octadienal and decadienal were found in freshwaters on the same level of concentration as in marine waters (Bartual and Ortega, 2013). It was noted that the concentration of octadienal and decadienal was evidently higher in water samples from Boduszewo. A very good correlation ($r = 0.93$) was found between the total aldehyde concentration and a value of absorbance at 254 nm. Positive correlation ($r = 0.61$) was also observed between PUAs concentration and turbidity. However, in the case of low value of turbidity, as was typical for water samples collected in Morasko (ca. 1.6 NTU), no positive correlation was noticed.

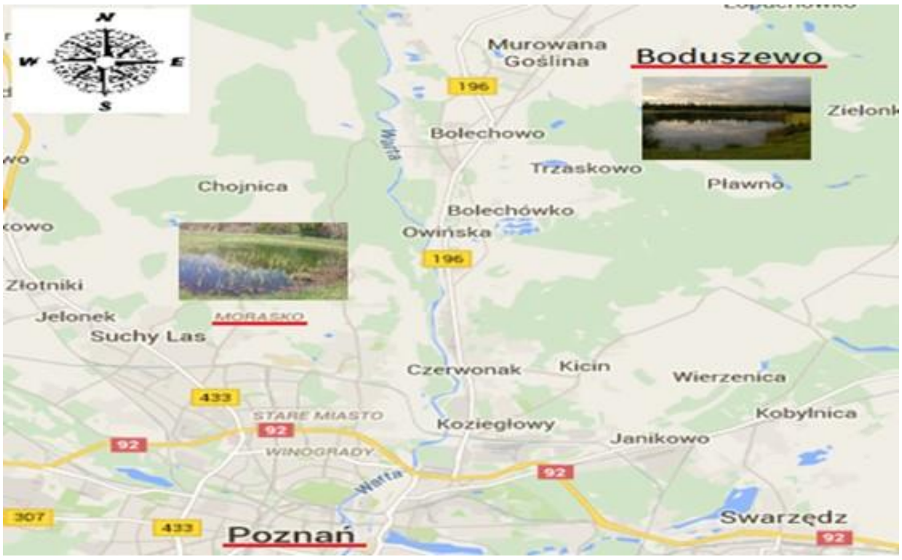

Figure 1. View of water reservoirs: A - artificial reservoir located on campus in Morasko B - natural pond located in rural area in Boduszewo

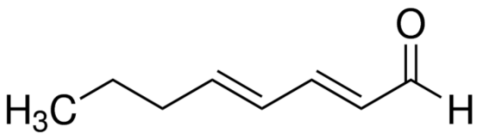	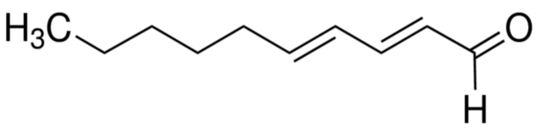
2,4-octadienal, 124.18 g/mol $CH_3CH_2CH_2CH=CHCH=CHCHO$ ($C_8H_{12}O$)	2,4-decadienal, 152.24 g/mol $CH_3(CH_2)_4CH=CHCH=CHCHO$ ($C_{10}H_{16}O$),

Figure 2. Structure of monitored polyunsaturated aldehydes

Water sample	Absorbance at 254 nm			Turbidity [NTU]			Dissolved oxygen [mg/l]		
	min.	max.	average	min.	max.	average	min.	max.	average
A	0.008	0.151	0.058	0.8	2.8	1.6	8.3	20.6	14.0
B	0.239	0.468	0.338	23.4	43.5	33.4	9.6	13.8	11.4

Table 1. Physico-chemical parameters of water samples: A - reservoir in Morasko, B - natural pond in Boduszewo

When turbidity is low the sun rays can penetrate more easily in the water. We have shown degradation of octadienal and decadienal affected by sun light, temperature, and bacteria. After one day the concentration of octadienal can decrease more than 25 % and after one week even more than 98 % (see Figure 4).

4. Conclusions

Polyunsaturated aldehydes are released into aquatic environment as bioactive secondary metabolites. PUAs have negative impact on algae grazers causing carcinogenic and mutagenic effect. The phenomenon of PUA emerging in marine waters was described by many authors. This study shows the presence of polyunsaturated aldehydes also in freshwaters. Octadienal and decadienal were found in freshwaters on the same level of concentration as in marine waters. PUAs are used as carbon source by bacteria. They can contribute to PUAs destruction under favorable conditions of environment (availability of light, appropriate temperature). The use of gas chromatograph in the system with selective electron capture detector has been proposed as a sensitive technique for PUAs determination.

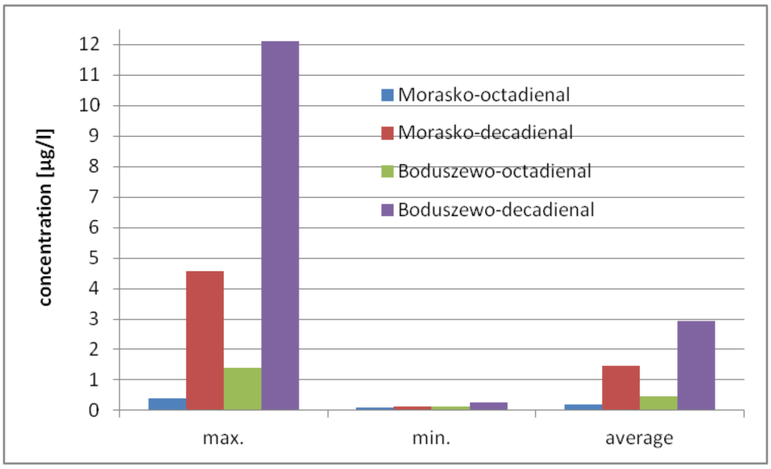

Figure 3. Range of PUA concentration in water samples from Morasko and Boduszewo

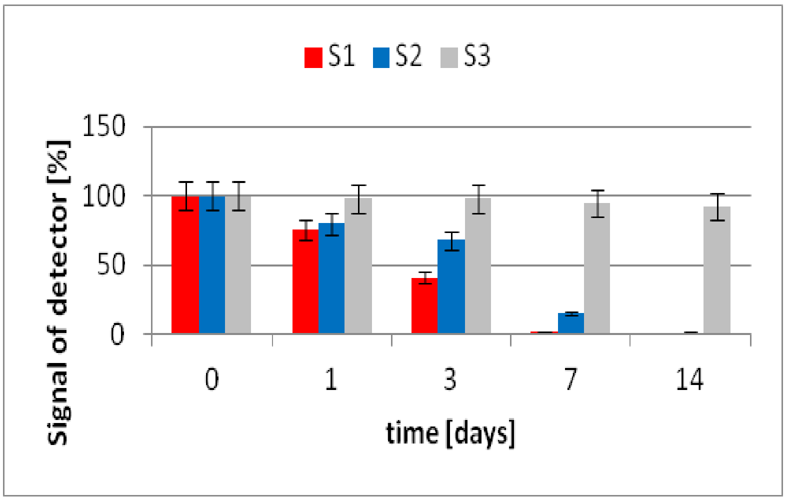

Figure 4. Degradation of octadienal. S1: effect of sun light, temperature of 20^{o}C and autochtonic bacteria; S2: effect of temperature of 20^{o}C and autochtonic bacteria; S3: effect of low temperature of 4 ^{o}C and biocide application

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