Radiocarbon, Vol 57, Nr 3, 2015, p 481–492 DOI: 10.2458/azu_rc.57.18358

$\Delta^{14}C$ AND $\delta^{13}C$ VARIATIONS IN ORGANIC FRACTIONS OF BALTIC SEA SEDIMENTS

Galina Lujanienė[1,2] • Jonas Mažeika[3] • Hong-Chun Li[4] • Rimantas Petrošius[1,3] •
Rūta Barisevičiūtė[1] • Kęstutis Jokšas[3,5] • Nijolė Remeikaitė-Nikienė[1,6] • Vitalijus Malejevas[1,6] •
Galina Garnaga[6] • Algirdas Stankevičius[1,6] • Ieva Kulakauskaitė[1] • Pavel P Povinec[7]

ABSTRACT. This article investigates variations of $\Delta^{14}C$ and $\delta^{13}C$ of total organic carbon (TOC) in sediments as well as in humic acids, lipid, and phospholipid fractions isolated from the surface (0–3 cm) sediment samples collected in the Curonian Lagoon and in the Baltic Sea. This study was performed to estimate relative contributions of the marine and terrestrial inputs to organic carbon in sediments, to assess a possible effect of petroleum hydrocarbon contamination on radiocarbon signatures, and to elucidate a possible leakage of chemical warfare agents (CWA) at the Gotland Deep dumpsite. Depleted $\Delta^{14}C$ values of the TOC (down to –453‰) and of the total lipid extracts (down to –812.4‰) were detected at the CWA dumpsite. Application of the compound-specific method indicated a possible effect of CWA on depleted $\Delta^{14}C$ and $\delta^{13}C$ values in the investigated organic carbon fractions. The obtained results have indicated the different origin and behavior of lipids and TOC at the CWA dumpsite as compared to the area affected by the terrestrial-freshwater OC input. The $\Delta^{14}C$ data of the TOC and total lipid extracts showed that recent sediments at the CWA dumpsite contain an excess of fossil carbon capable of influencing the ^{14}C dating at the site.

INTRODUCTION

Marine ecosystems are final recipients of pollutants introduced by river runoff or via atmospheric deposition. The Baltic Sea is supposed to be more sensitive to contamination as compared to other marine systems because of its semi-closed nature and specific biochemical conditions. It has been exposed to a wide range of organic and inorganic pollutants, including chemical warfare agents (CWA). The primary production, river runoff, and inflow from the North Sea are considered the most important sources of organic carbon (OC) in the Baltic Sea (Jansen et al. 2003; Kuliński and Pempkowiak 2011). The annual riverine inflow of OC to the Baltic Sea exceeds 4 million tons. The main part of OC introduced to the Baltic Sea consists of dissolved OC mostly derived from terrestrial humic substances (HS), while particulate OC is usually a minor fraction thought mainly to consist of plankton biomass (Cai et al. 1998; Stedmon et al. 2007; Giani et al. 2010; Hoikkala et al. 2015).

It is well known that sediments are of major importance to the sink of various pollutants including OC derived from the mixture of autochthonous water column sources and allochthonous terrestrial sources. It has been estimated that the terrestrial OC significantly contributes to the sediments in the southern (41–73%) and northern (65–85%) Baltic Sea (Alling et al. 2008; Szczepanska et al. 2012). The OC from terrestrial sources in the sediments consists of lignins, phenols, phenolic acids, cellulose, and xylose, while the HS are the most abundant fractions (Jednačak-Bišćan and Juračić 1987; Moran and Hodson 1989; Stevenson 1994). The input of HS to the Baltic Sea was estimated from measurements carried out in the Vistula River water, showing that 24.1% (2.1 mg/L) of HS on average are present in the particulate OC. The average concentration of dissolved OC in the Vistula water was found to be 7.1 mg/L with a comparatively high percentage (56.0%) of dissolved HS in the dissolved organic carbon (DOC) (Kuliński and Pempkowiak 2011). The flocculation of dissolved OC and sedimentation processes are responsible for the sink of OC (Deutsch et al. 2012; Asmala et al. 2014). The cycling of OC plays an important role in redistribution and bioavailability of trace elements. The OC serves as a source of energy and nutrients for algae, plankton, and bacte-

---

1. SRI Center for Physical Sciences and Technology, Vilnius, Lithuania.
2. Corresponding author. Email: lujaniene@ar.fi.lt.
3. SRI Nature Research Centre, Vilnius, Lithuania.
4. NTUAMS Laboratory at National Taiwan University, Taipei, Taiwan.
5. Vilnius University, Vilnius, Lithuania.
6. EPA Department of Marine Research, Klaipeda, Lithuania.
7. Comenius University, Bratislava, Slovakia.

Proceedings of the 1st International *Radiocarbon in the Environment* Conference
18–22 August 2014, Queen's University Belfast, Belfast, Northern Ireland, UK
Edited by Evelyn Keaveney and Paula Reimer

ria, and affects the acid-base balance in natural waters as well (Ulfsbo et al. 2015).

Knowledge about cycling of OC is important for understanding biogeochemical processes, stability of ecosystems, and prediction of human impacts on global climate change. It has been recognized that estuaries can supply a huge amount of organic substances to the marine environment. Despite several decades of studies of OC in the Baltic Sea, there is a lack of information about sources, distribution, and sink of OC in the Curonian Lagoon–Baltic Sea system. Although the Nemunas River and the Curonian Lagoon discharges heavily contribute to the sedimentary material, the sources of OC in sediments of the Lithuanian economic zone of the Baltic Sea have not been estimated yet.

It is generally accepted that analysis of $\delta^{13}C$ is an excellent tool to distinguish between terrestrial and marine OC sources (Mook and Tan 1991; Schubert and Calvert 2001). It has been found that in spite of the dissolved OC losses of >50% (due to sedimentation processes in the coastline), the inflow of the terrestrial OC has a substantial effect on the Baltic Sea ecosystem (Deutsch et al. 2012). The composition of complex OC mixtures can be resolved by analysis of biomarkers, e.g. via application of phospholipid-derived fatty acids (PDFA) biomarkers in marine plankton algae, in bacteria and land plants. Although these biomarkers accounted for only ~1% of the TOC in sediments of the Baltic Sea (Pazdro et al. 2001), compound-specific $\delta^{13}C$ and $\Delta^{14}C$ analyses of biomarkers are very useful for the source characterization since the responses of microbial communities to contamination of sediments by organic matter are generated via changes in composition and concentration of biomarkers (Uchida et al. 2001; Mollenhauer and Rethemeyer 2009; Druffel et al. 2010; Keaveney et al. 2015 [this issue]).

The aim of the present study has been to fill existing gaps on the sources and fate of OC in the coastal and open Baltic Sea, to assess possible effects of petroleum contamination on ^{14}C signatures, and to elucidate a possible leakage of CWA at the dumping site. It is rather difficult to assess CWA agents using only chromatographic/mass spectrometry methods since there are several compounds present in CWA. Degradation rates of these compounds and of their degradation products, as well as their stability in the marine environment, are not well known. CWA were produced before World War II from petroleum, natural gas, and petroleum distillates and from coal (Soborovsky and Epstein 1938; Franke 1973). For this reason, variations in carbon concentrations and in carbon isotope ratios have been expected. It has been supposed that applications of carbon isotopes will allow us estimate the total amount of CWA released into environment. No other known method can provide such a result. This study constitutes a first attempt to apply stable and radioactive carbon isotopes for CWA leakage studies.

EXPERIMENTAL

Sediment Sampling

The sampling stations in the studied area are shown in Figure 1. The area is characterized by horizontal and vertical salinity gradients from 0.5 to 7‰ and from 5 to 9‰, respectively, as well as by a variable loading with particulate suspended matter. The Curonian Lagoon is mainly a freshwater body with salinity of about 0.5‰. The intrusions of the Baltic Sea water to the Curonian Lagoon via the Klaipeda Strait due to fluctuations of the water level and hydrometeorological conditions can cause an increase in salinity from 0.5 to 8‰. Details on the sampling area and sediment characterization are given in previous publications (Lujanienė et al. 2005, 2013; Remeikaitė-Nikienė et al. 2012). Sediments were collected using a Van Veen grab during sampling campaigns in the framework of the state monitoring programs in 2012–2014, as well as during two expeditions to the CWA dumping site located in the Gotland Deep (Figure 1), which were carried out in 2013 and 2014. The

Ekman-Birge sampler with an effective grasping area of 225 cm^2 and weight of 3.5 kg was used for sampling in the Curonian Lagoon. Values of δ^{13}C were measured in all samples, and Δ^{14}C was measured in all samples except those with low TOC content.

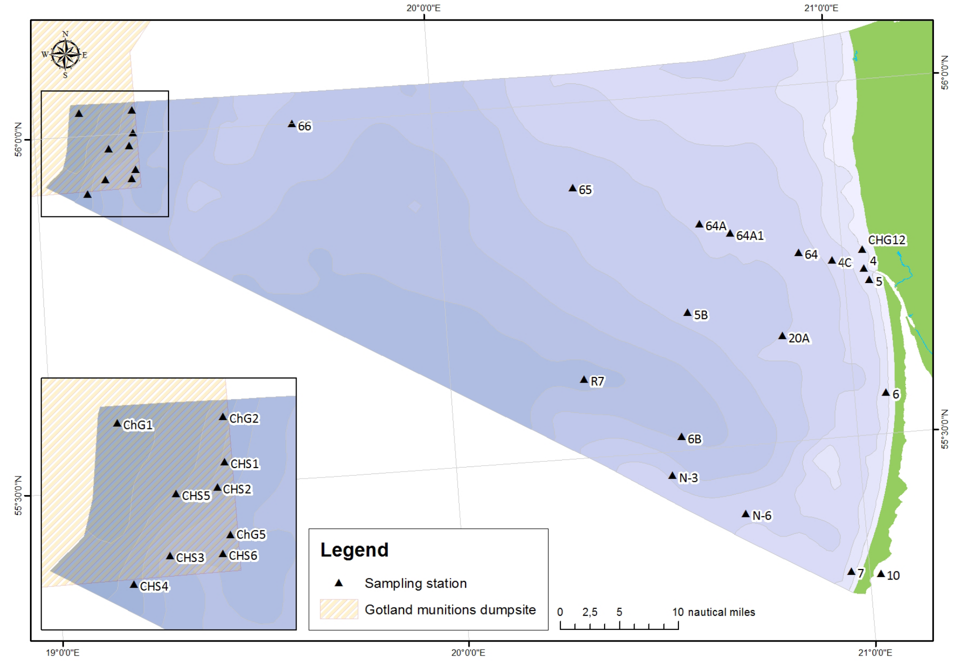

Figure 1 Sampling locations in the Curonian Lagoon and the Baltic Sea

Methods

Extraction of lipids from freeze-dried, homogenized surface sediments (0–3 cm) was performed according to published techniques (Zelles 1999; Tiwari et al. 2011). Total lipid extracts (TLE) were extracted using methylene chloride, methanol, and phosphate buffer mixture. The TLE were gently evaporated under the nitrogen stream at 37°C and stored at –20°C until further analysis. After removal of elemental sulfur, neutral lipid (NL), glycolipid, and phospholipid fractions for δ^{13}C measurements were separated by an SPE-SI column (following Zelles 1999; Tiwari et al. 2011). The phospho-(polar) lipids were esterified with methanol and KOH to produce fatty acid methyl esters (FAME), which were passed through a SPE-NH2 column to separate unsubstituted FAME. The mixture then was passed through an SPE-SCX (Supelco, USA) to separate saturated, monosaturated, and polyunsaturated PDFA.

Concentrations of total petroleum hydrocarbons (THC) and their two fractions—diesel (C_{10}–C_{20}) hydrocarbon (DHC) and mineral oil (C_{20}–C_{40}) hydrocarbon (MOHC)—were measured in sediment samples collected in 2013. The THC was extracted from freeze-dried and homogenized sediments using mechanical shaking (200 rpm) with a mixture of n-hexane and methylene chloride (1:1). The extracts were then purified using Massachusetts EPH SPE cartridges (Resprep), concentrated down to a volume of 1 mL in the rotary evaporator, and analyzed with the GC-FID. Humic substances were separated using the conventional alkali extraction (1 mol/L NaOH) method, and then humic acids (HA) with HCl at pH 1.0 and mineral fractions with 0.3 mol/L K^+ were separated (Stevenson 1994; Swift 1996). Since the mineral fraction contained HA, it was interpreted as the humic acid mineral fraction (HAMF) and used for further analyses to estimate the origin of the HA.

Measurements

The sediment samples were acidified with HCl to remove carbonates, dried at 60°C overnight, then weighed and placed into tin cups. The content and isotopic ratio of carbon in the samples were measured at the State Research Institute Center for Physical Sciences and Technology (Vilnius, Lithuania) using a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Flash EA 1112 elemental analyzer. Results of isotopic ratios were reported according to conventional standards, i.e. V-PDB for carbon, defined as

$$\delta^{13}C = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3 \text{ (‰)}$$

where R = $^{13}C/^{12}C$. For calibration of the reference gas (CO_2), the international standards from the International Atomic Energy Agency (Vienna) were used. IAEA-600 and NBS-22 standards were used during ^{13}C analyses. Repeated analyses of homogeneous material yielded a standard deviations for carbon of less than 0.08‰. At operational temperatures (250–260°C), no column bleeding was observed. Blanks were prepared and run for all types of chemical processing. The samples were run only when repeated analysis of 0.1 mM methyl palmitate ($n = 4$; injection volume was 0.2 mL) yielded standard deviations below 0.3%.

The $\delta^{13}C$ measurements of FAME were carried out with the Finnigan Trace GC ultra gas chromatograph, combined with the Thermo Finnigan Delta plus Advantage stable isotope spectrometer. The column used for the FAME measurement was a Supelco SP^{TM}-2380 capillary column of 0.25-mm internal diameter, 30 m in length, and of 0.2-μm film thickness. Helium was used as a carrier gas with a constant flow rate of 1.4 mL min^{-1}. The injector had a temperature of 250°C. The time-temperature program for the oven was as follows: initial temperature at 50°C for 5 min, then increase by 2°C min^{-1} until 260°C for 20 min. Separated FAME was converted to CO_2 in an oxidation reactor at 950°C. After removal of water through a Nafion dryer, CO_2 then entered the IRMS. The $\delta^{13}C$ values were corrected using a laboratory working standard of methyl palmitoleate from Fluka. It was calibrated to IAEA-600 and IAEA-CH-3 using NBS19 and NBS20 using the Flash EA 1112 elemental analyzer inlet of the Scientific Delta V Advantage mass spectrometer with SD = ±0.1‰. This standard was analyzed separately from the samples during isotopic analyses of FAME. Repeated analyses of this material yielded SD <0.2‰. (The smallest detectable peaks were ~6 ng C.) TOC was measured using the LQUI TOC analyzer. Separate $\delta^{13}C$ measurements were carried out on all ingredients added to investigated samples (e.g. methanol), and final results were corrected for these contributions.

The gas chromatography with flame ionization detector (GC-FID) technique was used for the identification and quantification of the diesel and mineral oil fractions and aliphatic hydrocarbons in the collected sediment samples [ISO 16703:2004(E), International Organization for Standardization 2004]. The GC was equipped with a Restek Rxi-1ms column. Helium was used as a carrier gas at a constant velocity of 40 cm s^{-1}. The temperature of the column was held at 60°C for 5 min, then increased to 315°C at a rate of 10°C min^{-1}, and held for 20 min. The compound identification was based on GC retention times of authentic standards, injected and analyzed under the same conditions as the samples. A standard mixture containing two types of oil—diesel fuel (type A) and mineral oil (type B) in hexane (Restek)—was used for identification of diesel and mineral oil fractions in the analyzed samples. The system performance was checked using the System Performance Test Standard Mixture of n-alkanes in hexane.

Measurements of $\Delta^{14}C$ in sediments and in different classes of organic substances were carried out using a 1.0MV HVE Tandetron AMS in the Department of Geosciences of the National Taiwan

University (Gottdang et al. 1995). All samples were pretreated free of carbonate; each sample was placed in a quartz tube with ~70 mg of CuO and a silver piece, then put on the vacuum line to pump out air until 10^{-3} mbar. The quartz tube was heated to 850°C to combust OC into CO_2. The CO_2 was purified and sealed into a glass tube. The purified CO_2 was introduced into the graphitization system, which contains two quartz tubes with Zn and Fe powders separately under vacuum of 10^{-3} mbar. The CO_2 was converted to graphite with Fe (Fe:C = 3.5:1) under 550°C for 6–8 hr while about 400 mg Zn under 450°C was used for catalysis. Graphite samples were pressed into targets and measured for their $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios with oxalic acid standard (OXII, 4900C; National Institute of Standards and Technology, Gaithersburg, USA) and background (NTUB and CWOC) targets at the Accelerate Mass Spectrometer Radiocarbon Dating Laboratory of the National Taiwan University (lab code NTUAMS). The standard and background samples were subjected to the same procedures as the sediment samples to make graphite targets. Every batch of samples included at least three OXII standards and three backgrounds. The $\Delta^{14}C$ values were calculated by the following equation:

$$\delta^{14}C = [(^{14}C_{\text{sample}} - {}^{14}C_{\text{standard}})/{}^{14}C_{\text{standard}}] \times 10^3 \text{ (‰)}$$

where $^{14}C_{\text{standard}}$ is corrected for decay from year of measurement to 1950

$$\Delta^{14}C = \delta^{14}C - 2(\delta^{13}C + 25)(1 + \delta^{14}C/1000) \text{ (‰)}$$

The $\delta^{13}C$ is the $^{13}C/^{12}C$ ratio measured by the AMS simultaneously with the ^{14}C measurement (Kim et al. 2001). The counting uncertainty of ^{14}C for the OXII standard is generally <0.3% (1σ) and the standard deviation of $\Delta^{14}C$ for repeated OXII standard measurement is about 1‰ (1σ).

RESULTS

Sediments

The spatial distribution of TOC, $\delta^{13}C$ of TOC, $\Delta^{14}C$ of TOC, THC, DHC, and MOHC in the sediments of the studied region is shown in Figure 2. The TOC found in Baltic Sea sediments varied from 0.1% to a maximum found at station R7 (7.22%). The Curonian Lagoon value for station CL10 was even higher (10.06%). The station CL10 station is located in the accumulation zone in the Curonian Lagoon, and it is the most representative station, also used in comparisons. The main source of TOC in the area is the Curonian Lagoon, and the plume could affect the isotopic ratios. However, no strong correlation was observed for TOC with $\delta^{13}C$ and $\Delta^{14}C$ values. The Pearson correlation and significance for the Baltic Sea sediments were as follows: $\delta^{13}C$ ($r = 0.15$, $p = 0.57$); $\Delta^{14}C$ ($r = 0.29$, $p = 0.23$). For the CWA dumping site, the values were $\delta^{13}C$ ($r = -0.32$, $p = 0.002$) and $\Delta^{14}C$ ($r = -0.27$, $p = 0.52$). The high values of TOC and low $\delta^{13}C$ values found at station R7 in the Baltic Sea can be explained by a different pattern of water circulation since this station is located in the accumulation zone at the Nemunas River crease. A different pattern of contaminant behavior at this station was found in previous studies as well (Lujanienė et al. 2005, 2014).

The spatial distribution of THC, DHC, and MOHC in the investigated area has a similar pattern since the TPH is the sum of DHC and MOHC (Figure 2). Concentrations of THC varied from 4.847 to 67.51 mg kg^{-1}, and the highest concentrations were found at sampling stations 20A and 5B in the Baltic Sea (Figure 1). Station 20A is located in the area of the dumpsite where recent sediments (glacial morainic mud and mud) from the Klaipėda port were dumped at ~45 m depth. The dumping area covers 17.8 km^2, and the annual amount of dredged sediments exceeds 2 Mm^3. The dredged sediments consist of a mixture of recent surface sediments with usually high contents of OC, largely diluted with old sediments from up to 2 m depth with a low organic content. For this reason, the TOC in the sediments is low. The performed monitoring revealed slowly increasing deformations of the sea bottom at the dumpsite, and sediment transport towards station 5B explaining higher THC concentrations detected at this station. Thus, the petroleum hydrocarbons might be introduced by the sediment dumping from the Klaipėda port. However, the enhanced THC concentration at station ChS1 was rather unexpected since at all CWA dumpsite stations (except station ChS6 with sand-silty sediments), mud silty-coarse grained sediments were found.

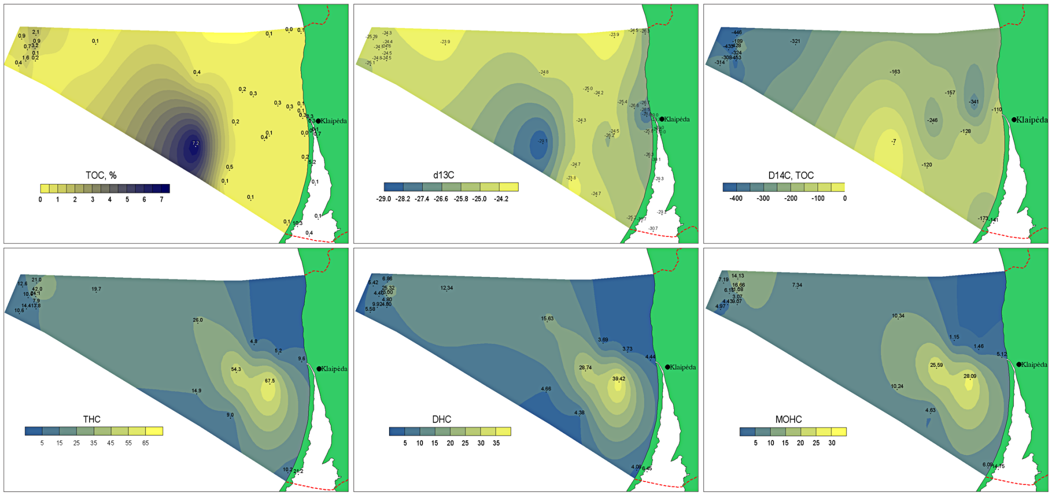

Figure 2 Spatial distribution of TOC, %; δ^{13}C of TOC, ‰; Δ^{14}C of TOC, ‰, THC, mg/kg; DHC, mg/kg; MOHC, mg/kg in the sediments in the studied area.

^{14}C and ^{13}C of Total Organic Carbon (TOC) and Total Lipid Extracts (TLE)

Δ^{14}C values of the TOC and the TLE extracted from sediments as a function of water depth are shown in Figure 3A. The TOC and TLE values in sediments ranged from –446.1‰ to –6.7‰ and from –812.4‰ to –102.9‰, respectively. Generally, the Δ^{14}C of TLE has shown depleted values (down to –800‰) and larger variations when compared with TOC. The depleted Δ^{14}C values of TLE as compared to TOC were observed in previous publications as well (e.g. Druffel et al. 2010). This is probably related to the microbiological activity and utilization of dissolved organic carbon (DOC), which is then usually more depleted in seawater (Druffel and Bauer 2000). The most depleted $\Delta^{14}C_{TOC}$ and $\Delta^{14}C_{TLE}$ values in sediments were observed at the sampling stations located at the CWA dumpsite (Figure 1). The dumped CWA were produced before World War II from petroleum, natural gas, petroleum distillates, and coal, i.e. from fossil sources. Therefore, ^{14}C concentrations show more depleted values, and to a lesser extent, changes in stable isotope ratios could be expected. The $\Delta^{14}C_{TOC}$ and $\Delta^{14}C_{TLE}$ values differed by a factor of 4. While the depleted $\Delta^{14}C_{TOC}$ and $\Delta^{14}C_{TLE}$ values were expected at the CWA dumpsite, low $\Delta^{14}C_{TLE}$ values measured in the samples collected at sampling stations 7 and 6B were rather surprising and difficult to explain. A comparatively low amount of TOC was found at these stations: 0.12 and 0.46%, respectively. In this case, even a small amount of OC from petroleum, resuspension, erosion, or submarine groundwater discharges (Szymczycha et al. 2014) could affect the Δ^{14}C values. However, most probably these low values are due to an oil contamination, when various synthetic compounds produced from petroleum and widely used detergents and pharmacy products, could either directly or indirectly (after their utilization by microorganisms) affect the isotopic composition of DOC. These contaminants can be transported with the Nemunas River waters via the Curonian Lagoon to the Baltic Sea. The effect of the Curonian Lagoon plume can be illustrated by a spatial distribution of δ^{13}C with low values observed in the coastal zone of the Baltic Sea (Figure 2). Although low $\Delta^{14}C_{TLE}$ values were measured in the coastal zone sampling at station 7 and in the open waters at stations 6b and 66 (contrary to station R7 in the Baltic Sea and the Curonian Lagoon station CL10), it is supposed that their sources at the CWA dumping site are different. At the CWA dumpsite, the average depth is 92 m (from 78 to 106 m) and the $\Delta^{14}C_{TOC}$ values varied from –453‰ to –189‰, with an average value of –362 ± 4‰ ($n = 8$), while $\Delta^{14}C_{TLE}$ values varied from –812‰ to –261‰, with an average value of –599 ± 15‰ ($n = 8$). At the shallower sampling stations, with an average depth of 40 m (from 5 to 72 m), the $\Delta^{14}C_{TOC}$ varied from –6.7‰ to –341‰ with an average value of –176 ± 18‰ ($n = 10$). These values are comparable with that found at station CL10 (–141 ± 14‰) in the Curonian Lagoon. We did not find any correlation between the $\Delta^{14}C_{TOC}$ and $\Delta^{14}C_{TLE}$ values.

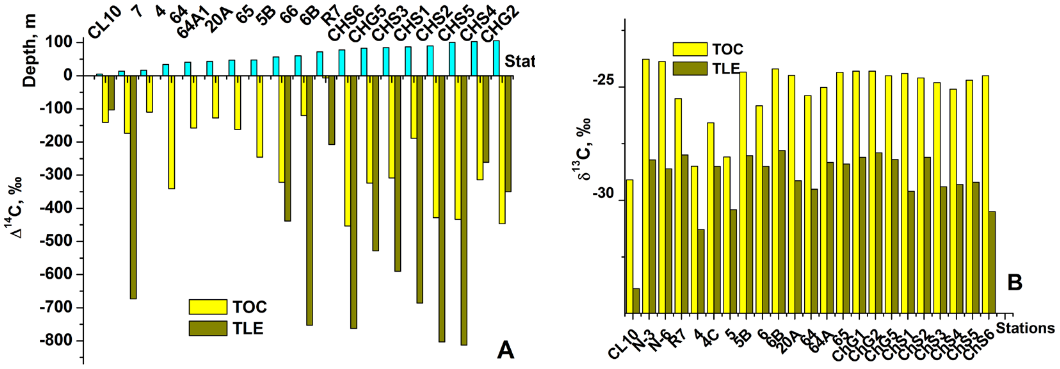

Figure 3 (A) $\Delta^{14}C$ of TOC and TLE extracted from aediments vs. water depth. (B) $\delta^{13}C$ of TOC and TLE in bottom sediments.

TOC and TLE $\delta^{13}C$ values varied from –29.1‰ to –23.8‰ and from –33.9‰ to –27.8‰, respectively (Figure 3B). There were no large variations (or differences) between TOC and TLE with the exception of sampling station CL10 located in the Curonian Lagoon, and sampling stations 4 and 5 in the Baltic Sea located close to the Klaipeda Strait (Figure 1) where the most depleted $\delta^{13}C_{TOC}$ and $\delta^{13}C_{TLE}$ values were detected. Contrary to the $\Delta^{14}C$ data, $\delta^{13}C_{TOC}$ values correlated well with $\delta^{13}C_{TLE}$ values ($r = 0.76$, $p = 0.00004$), and showed (on the average) a difference only by a factor of 1.15.

$\Delta^{14}C_{TOC}$ values of THC, DHC, and MOHC fractions in sediments of the Curonian Lagoon (station CL10) and in the open Baltic Sea are compared in Figure 4. No correlation was observed between $\Delta^{14}C_{TOC}$ and THC (Pearson correlation, significance: THC $r = 0.17$, $p = 0.48$; DHC $r = 0.17$, $p = 0.48$; MOHC $r = 0.16$, $p = 0.52$).

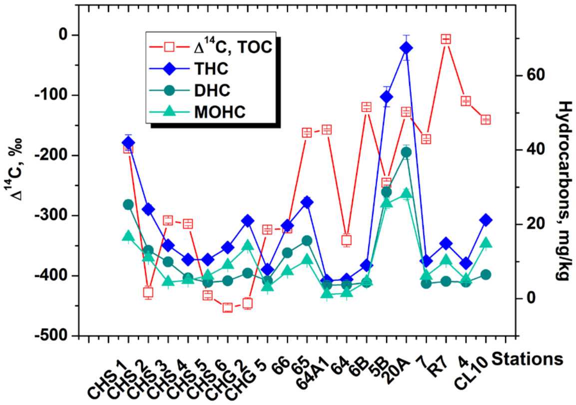

Figure 4 $\Delta^{14}C$ in total organic carbon (TOC), total petroleum hydrocarbons (THC), diesel hydrocarbon (DHC), and mineral oil hydrocarbon (MOHC) fractions in sediments of the Curonian Lagoon (station CL10) and in the open Baltic Sea.

The $\delta^{13}C_{TOC}$, $\delta^{13}C_{HA}$, $\delta^{13}C_{TLF}$, and $\delta^{13}C_{HAMF}$ values of sediment extracts are presented in Figure 5. The $\delta^{13}C_{TOC}$ and $\delta^{13}C_{HA}$ values varied between –29‰ and –24‰, usually with lower values of $\delta^{13}C_{TLF}$ (from –33.9‰ to –27.8‰). There are small differences between $\delta^{13}C_{HA}$ and $\delta^{13}C_{HAMF}$, except at stations 65 and ChG5 (the most enriched $\delta^{13}C_{HAMF}$ value was –17.3‰) located in the open Baltic Sea, which may be due to the OC preservation in clay minerals. The $\delta^{13}C_{TOC}$ values correlated well with that of $\delta^{13}C_{HA}$ ($r = 0.98, p = 0.000009$), $\delta^{13}C_{TLF}$ ($r = 0.87, p = 0.005$) and $\delta^{13}C_{HAMF}$ ($r = 0.68, p = 0.06$).

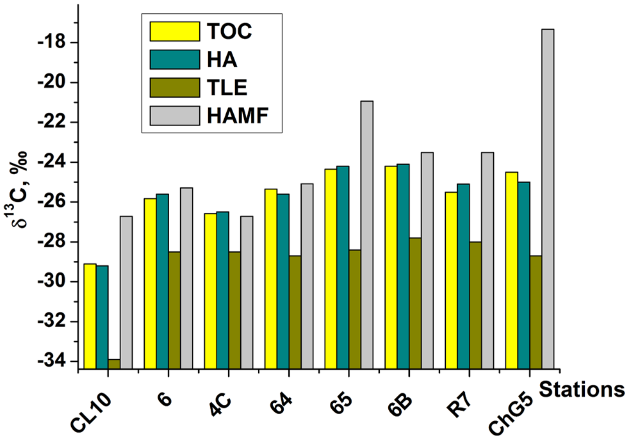

Figure 5 δ^{13}C values of total organic carbon (TOC), total lipid extracts (TLE), humic acids (HA), and humic acid mineral fraction (HAMF) extracted from sediments.

Variations of δ^{13}C values in neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions isolated from sediments showed small differences between the investigated fractions—from –31.7‰ to –27.8‰, from –30.9‰ to –27.3‰, and from –31.1‰ to –26.7‰, respectively (Figure 6A).

The δ^{13}C values in PDFA (C14:0, C16:0, C16:1w7c, C18:1w9c, and C18:2) ranged from –37.6‰ to –28.6‰, from –31.5‰ to –26.0‰, from –29.3‰ to –26.1‰, from –33.9‰ to –27.1‰, and from –31.3‰ to –25.1‰, respectively (Figure 6B). The most depleted δ^{13}C values were found for the C14:0 biomarker in sediments collected at sampling stations ChS2 (–37.6‰), ChS5 (–36.0‰), and ChS1 (–33.4‰) at the CWA dumpsite. A rather low δ^{13}C value of biomarker C18:1w9c (–33.9‰) was measured in sediments at station ChS1. Generally, all markers showed more depleted values in samples from the CWA dumpsite. In addition, biomarker C18:2 was not detected at the CWA site. The probable source of biomarkers C16:0 and C16:1w7c are microalgae and bacterial (e.g. Gramm negative) biomass. It is well known that algae, zooplankton, and cyanobacteria are enriched in C18 unsaturated FA, while both C16:0 and C18:1w9c were dominant in Cyanophyceae and Chlorophyceae species (Volkman et al. 1998, 2008; Wakeham et al. 2007; Sahu et al. 2013). All PDFA used in this study have a low preservation potential and can reflect the most recent events. Biomarker C14:0 shows the presence of phytoplankton and presumably indicates the fresh marine source since short-chain FAs have a higher degradation rate in the marine environment (Sun and Wakeham 1994).

However, both activities of methane oxidizing bacteria and leakage of CWA could decrease the Δ^{14}C and δ^{13}C values. Specific biomarkers could help to distinguish between methane oxidizing bacteria and CWA sources. PDFA were used as biomarkers in this study because bacterial activities and higher arsenic concentrations were detected at the CWA dumpsites (Garnaga et al. 2006; Medvedeva et al. 2009). Microbiological studies performed at the CWA dumping sites in the Baltic Sea showed an increased activity of mustard gas hydrolysis products degrading bacteria (*Achromobacter* sp., *Pseudomonas* sp., and *Arthrobacter* sp.) in the near-bottom water capable of utilizing the major product of hydrolysis (Medvedeva et al. 2009) in the marine environment at low temperature.

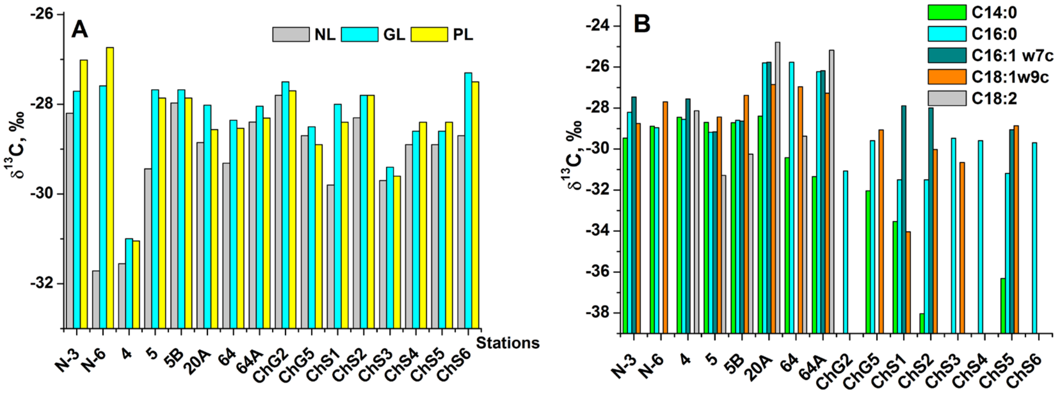

Figure 6 (A) Variations of δ^{13}C values in neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions isolated from bottom sediments. (B) Variations of δ^{13}C values in phospholipid-derived FAs: C14:0, C16:0, C16:1w7c, C18:1w9c, and C18:2 (B).

Therefore, the response of bacteria, phytoplankton, and zooplankton to the contamination via variations in isotopic signatures was expected. The biomarkers used in this study have a low preservation potential; thus, they can indicate the most recent events. None were identified as a methane oxidation biomarker. Lipid biomarkers for aerobic methanotrophy in the Gotland Deep (Baltic Sea) and anaerobic oxidation of methane and sulfate reduction in cold seep sediments of Nyegga pockmarks (Norwegian Margin) have been reported to be as follows: C16:1w8c, C16:1w5c, aiC15:0, 10Me-C16:0, and C18:2 FAs (w7c,12c and w6c,12c). The δ^{13}C-depleted values were found in biomarkers: down to –41.2‰ for C16:1w8c and C16:1w5c (Bodelier et al. 2009; Schmale et al. 2012; Berndmeyer et al. 2013; Chevalier et al. 2014). Analyses of individual FA collected in water samples at 100 m water depth in the Gotland Deep indicated specific biomarkers for the methanotrophic bacteria, C16:1w8c and C16:1w5c. The depleted values of δ^{13}C for these biomarkers were found to be –38.8‰ and –35.7‰, respectively. Along with these biomarkers, δ^{13}C values were measured in C14:0, C16:0, C16:1w7c, C18:1w9c, and C18:2. No depleted δ^{13}C values in these biomarkers were detected; they ranged from –27.6‰ to –25.4‰ (Schmale et al. 2012). For this reason, we can assume that a source of low δ^{13}C values detected at the CWA dumpsite is not related to the activity of methanotrophic bacteria but most probably due to the release of the CWA to the marine environment. The measurements performed at the Gotland Deep CWA dumpsite in the framework of the CHEMSEA (http://www.chemsea.eu/) project identified the following chemicals: sulfur mustard (both intact and degradation products), adamsite (degradation products), Clark I (degradation products), phenyldichloroarsine (degradation products), triphenylarsine (intact and oxidized), and chloroacetophenone (intact). However, only in one sample from 10 taken at the same dumpsite in the Lithuanian economic zone were arsenic-containing CWAs detected (CHEMSEA). The sample was collected at station ChS5 where the most depleted Δ^{14}C TLE value (–812.4‰) was measured. Among other important findings of the CHEMSEA project, high concentrations of the mustard bacterial degradation product were found at the Gdansk Deep dumping site.

CONCLUSIONS

Data obtained in this study have indicated wide OC variations in the coastal and open Baltic Sea. The spatial distribution of $\delta^{13}C_{TOC}$ in coastal sediments showed strong impacts of terrestrial and fresh waters on OC. Similar values of $\delta^{13}C_{TOC}$ and $\delta^{13}C_{HA}$ revealed that humic acids (due to their high abundance) determine the δ^{13}C distribution in sediments. However, $\Delta^{14}C_{TLE}$ and $\delta^{13}C_{TLE}$ variations have shown mixed (relict and modern) sources of lipids in the coastal area. The most depleted values were detected for the $\Delta^{14}C_{TOC}$ (–453‰) and $\Delta^{14}C_{TLE}$ (–812.4‰) at the CWA dumping site in the Gotland Deep. The contribution of petroleum to the fossil fractions was estimated from measurements of THC in sediments. It was found that THC accounted for 0.81% of TOC, dry weight (from 0.02 to 5%). To investigate the reason for the high contribution of fossil sources (causing low $\Delta^{14}C$ values of TOC and TLE), as well as to distinguish between the methane oxidizing bacteria and CWA sources at the dumpsite, $\delta^{13}C$ compound-specific analyses were applied. The $\delta^{13}C$ analyzed in PDFAs (C14:0, C16:0, C16:1w7c, C18:1w9c, and C18:2) showed the most depleted $\delta^{13}C$ values for the C14:0 biomarker collected at sampling stations ChS2 (–37.6‰), ChS5 (–36‰), and ChS1 (–33.4‰) at the CWA dumpsite.

Although the data presented in this study indicated a possible effect of CWA on depleted $\Delta^{14}C$ and $\delta^{13}C$ values, additional studies are required to confirm these preliminary results. $\Delta^{14}C$ compound-specific analyses are necessary to distinguish between the methanotrophic and CWA sources at the CWA dumping site.

ACKNOWLEDGMENTS

Financial support provided by the Research Council of Lithuania (contract No. MIP-080/2012) is acknowledged. We are grateful for funding support to H-C Li for the AMS ^{14}C measurements from NSC 102-2811-M-002-177 and MOST 103-2116-M-002-001 of Taiwan. PPP acknowledges support provided by the EU Research and Development Operational Program funded by the ERDF (project No. 26240220004).

REFERENCES

Alling V, Humborg C, Mörth C-M, Rahm L, Pollehne F. 2008. Tracing terrestrial organic matter by $\delta^{34}S$ and $\delta^{13}C$ signatures in a subarctic estuary. *Limnology and Oceanography* 53(6):2594–602.

Asmala EDG, Bowers R, Kaartokallio AH, Thomas DN. 2014. Qualitative changes of riverine dissolved organic matter at low salinities due to flocculation. *Journal of Geophysical Research Biogeosciences* 119(10):1919–33.

Berndmeyer Ch, Thiel V, Schmale O, Blumenberg M. 2013. Biomarkers for aerobic methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea). *Organic Geochemistry* 55:103–11.

Bodelier PLE, Gillisen M-JB, Hordijk K, Damsté JSS, Rijpstra WIC, Geenevasen JAJ, Dunfield PF. 2009. A reanalysis of phospholipid fatty acids as ecological biomarkers for methanotrophic bacteria. *The ISME Journal* 3:606–17.

Cai W-J, Wang Y, Hodson RE. 1998. Acid–base properties of dissolved organic matter in the estuarine waters of Georgia, USA. *Geochimica et Cosmochimica Acta* 62(3):473–83.

Chevalier N, Bouloubassi I, Stadnitskaia A, Taphanel M-H, Damsté JSS. 2014. Lipid biomarkers for anaerobic oxidation of methane and sulphate reduction in cold seep sediments of Nyegga pockmarks (Norwegian margin). *Geo-Marine Letters* 34(2–3):269–80.

Deutsch B, Alling V, Humborg C, Korth F, Mörth CM. 2012. Tracing inputs of terrestrial high molecular weight dissolved organic matter within the Baltic Sea ecosystem. *Biogeosciences* 9:4465–75.

Druffel ERM, Bauer JE. 2000. Radiocarbon distributions in Southern Ocean dissolved and particulate organic matter. *Geophysical Research Letters* 27(10):1495–8.

Druffel ERM, Zhang D, Xu X, Ziolkowski LA, Southon JR, dos Santos GM, Trumbore SE. 2010. Compound-specific radiocarbon analyses of phospholipid fatty acids and n-alkanes in ocean sediments. *Radiocarbon* 52(2–3):1215–23.

Franke Z. 1973. Chemistry of warfare agents. *Chimija* 1:136. In Russian.

Garnaga G, Wyse E, Azemard S, Stankevičius A, de Mora S. 2006. Arsenic in sediments from the southeastern Baltic Sea. *Environmental Pollution* 144(3):855–61.

Giani M, Rampazzo F, Berto D. 2010. Humic acids contribution to sedimentary organic matter on a shallow continental shelf (northern Adriatic Sea). *Estuarine, Coastal and Shelf Science* 90(2):103–10.

Gottdang A, Mous DJW, van der Plicht J. 1995. The HVEE ^{14}C system at Groningen. *Radiocarbon* 37(2): 649–56.

Hoikkala L, Kortelainen P, Soinne H, Kuosa H. 2015. Dissolved organic matter in the Baltic Sea. *Journal of Marine Systems* 142:47–61.

International Organization for Standardization. 2004. ISO 16703:2004-11 (E). Soil quality - Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography. Geneva. p 1–18.

Jansen DL, Lundqvist DP, Christiansen Ch, Lund-Hansen LC, Balstrøm T. Leipe Th. 2003. Deposition of organic matter and particulate nitrogen and phosphorus at the North Sea–Baltic Sea transition – a GIS study. *Oceanologija* 45(1):283–303.

Jednačak-Bišćan J, Juračić M. 1987. Organic matter and surface properties of solid particles in the estuarine mixing zone. *Marine Chemistry* 22(2):257–63.

Keaveney EM, Reimer PJ, Foy RH. 2015. Young, old, and weathered carbon—Part 2: using radiocarbon and stable isotopes to identify terrestrial carbon support of the food web in an alkaline, humic lake. *Radiocarbon* 57(3):425–38. [this issue].

Kim J-C, Park J-H, Kim I-C, Lee C, Cheoun M-K, Kang J, Song Y-M, Jeong S-C. 2001. Progress and protocol at the Seoul National University AMS facility. *Journal of the Korean Physical Society* 39(4):778–82.

Kuliński K, Pempkowiak J. 2011. The carbon budget of the Baltic Sea. *Biogeosciences* 8:3219–30.

Lujanienė G, Garnaga G, Remeikaitė-Nikienė N, Jokšas K, Garbaras A, Skipitytė R, Barisevičiūtė R, Šilobritienė B, Stankevičius A, Kulakauskaitė I, Ščiglo T. 2013. Cs, Am and Pu isotopes as tracers of sedimentation processes in the Curonian Lagoon – Baltic Sea system. *Journal of Radioanalytical and Nuclear Chemistry* 296(2):787–92.

Lujanienė G, Jokšas K, Šilobritienė B, Morkūnienė R. 2005. Physical and chemical characteristics of ^{137}Cs in the Baltic Sea. *Radioactivity in the Environment* 8:165–79.

Lujanienė G, Remeikaitė-Nikienė N, Garnaga G, Jokšas K, Šilobritienė B, Stankevičius A, Šemčuk S, Kulakauskaitė I. 2014. Transport of ^{137}Cs, ^{241}Am and Pu isotopes in the Curonian Lagoon and the Baltic Sea. *Journal of Environmental Radioactivity* 127:40–9.

Medvedeva N, Polyak Y, Kankaanpää H, Zaytseva T. 2009. Microbial responses to mustard gas dumped in the Baltic Sea. *Marine Environmental Research* 68(2):71–81.

Mollenhauer G, Rethemeyer J. 2009. Compound-specific radiocarbon analysis – analytical challenges and applications. *IOP Conference Series*: *Earth and Environmental Science* 5:012006.

Mook WG, Tan FC. 1991. Stable carbon isotopes in rivers and estuaries. In: Degens ET, Kempe S, Richey JE, editors. *Biogeochemistry of Major World Rivers*. Chichester: John Wiley. p 245–64.

Moran MA, Hodson RE. 1989. Formation and bacterial utilization of dissolved organic carbon derived from detrital lignocellulose. *Limnology and Oceanography* 34(6):1034–47.

Pazdro K, Staniszewski A, Bełdowski J, Emeis K-Ch, Leipe Th, Pempkowiak J. 2001. Variations in organic matter bound in fluffy layer suspended matter from the Pomeranian Bay (Baltic Sea). *Oceanologija* 43(4):405–20.

Remeikaitė-Nikienė N, Lujanienė G, Garnaga G, Jokšas K, Garbaras A, Skipitytė R, Barisevičiūtė R, Šilobritienė B, Stankevičius A. 2012. Redistribution of trace elements and radionuclides in the Curonian Lagoon and the coastal zone of the Baltic Sea. Presented at the IEEE/OES Baltic 2012 International Symposium "Ocean: Past, Present and Future. Climate Change Research, Ocean Observations and Advanced Technologies for Regional Sustainability.

Sahu A, Pancha I, Jain D, Paliwal C, Ghosh T, Patidar S, Bhattacharya S, Mishra S. 2013. Fatty acids as biomarkers of microalgae. *Phytochemistry* 89:53–8.

Schmale O, Blumenberg M, Kießlich K, Jakobs G, Berndmeyer C, Labrenz M, Thiel V, Rehder G. 2012. Aerobic methanotrophy within the pelagic redoxzone of the Gotland Deep (central Baltic Sea). *Biogeosciences* 9:4969–77.

Schubert CJ, Calvert SE. 2001. Nitrogen and carbon isotopic composition of marine and terrestrial organic matter in Arctic Ocean sediments: implications for nutrient utilization and organic matter composition. *Deep-Sea Research I* 48(3):789–810.

Soborovsky LZ, Epstein GY. 1938. *Chemistry and Technology of Chemical Warfare Agents*. Moscow: GIOP, SSSR. In Russian.

Stedmon CA, Thomas DN, Granskog M, Kaartokallio H, Papadimitriou S, Kuosa H. 2007. Characteristics of dissolved organic matter in Baltic coastal sea ice: allochthonous or autochthonous origins? *Environmental Science & Technology* 41(21):7273–9.

Stevenson FJ. 1994. *Humus Chemistry: Genesis, Composition, Reaction*. New York: Wiley-Interscience.

Sun M-Y, Wakeham SG. 1994. Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea Basin. *Geochimica et Cosmochimica Acta* 58(16):3395–406.

Swift RS. 1996. Organic matter characterization. In: Sparks DL, Bartles JM, Bigham JM, editors. *Methods of Soil Analysis: Part 3. Chemical Methods*. Madison: Soil Science Society of America. p 1018–20.

Szczepańska A, Zaborska A, Maciejewska A, Kuliński K, Pempkowiak J. 2012. Distribution and origin of organic matter in the Baltic Sea sediments dated with ^{210}Pb and ^{137}Cs. *Geochronometria* 39(1):1–9.

Szymczycha B, Maciejewska A, Winogradow A, Pempkowiak J. 2014. Could submarine groundwater discharge be a significant carbon source to the southern Baltic Sea? *Oceanologija* 56(2):327–47.

Tiwari SC, Sureshkumar Singh S, Dkhar MS, Schloter M, Gattinger A. 2011. Microbial community structures of degraded and undegraded humid tropical forest soils as measured by phospholipid fatty acid [PLFA] profiles. *Journal of Biodiversity and Ecological Sciences* 1(3):197–212.

Uchida M, Shibata Y, Kawamura K, Kumamoto Y, Yoneda M, Ohkushi N, Harada M, Hirota M, Mukai, Tanaka A, Kusakabe M, Morita M. 2001. Compound-specific radiocarbon ages of fatty acids in marine sediments from the Western North Pacific. *Radiocarbon* 43(2B):949–56.

Ulfsbo A, Kuliński K, Anderson LG, Turner DR. 2015. Modelling organic alkalinity in the Baltic Sea using a Humic-Pitzer approach. *Marine Chemistry* 168:18–26.

Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F. 1998. Microalgal biomarkers: a review of recent research developments. *Organic Geochemistry* 29(5–7):1163–79.

Volkman JK, Revill AT, Holdsworth DG, Fredericks D. 2008. Organic matter sources in an enclosed coastal inlet assessed using lipid biomarkers and stable isotopes. *Organic Geochemistry* 39(6):689–710.

Wakeham SG, Amann R, Freeman KH, Hopmans EC, Jorgensen BB, Putnam IF, Schouten S, Damsté JS, Talbot HM, Woebken D. 2007. Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study. *Organic Geochemistry* 38(12):2070–97.

Zelles L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils* 29(2):111–29.