Bioavailability of Organic Contaminants in a Changing Climate

PhD. Thesis
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Environmental Technology, Norwegian University of Life Sciences

Abstract

The effects of predicted future climate change was investigated with special emphasis on the association of organic contaminants with dissolved organic carbon (DOC) in the Baltic Sea.

An automated method was developed for the measurements of DOC-water distribution constants at realistic DOC concentrations in brackish water. The method proved to be valid for 30 organic contaminants with different structural elements in the 5 – 100 mg carbon/L DOC concentration range. There were limitations of this method. Firstly, its applicability is limited towards contaminants with lower affinity to DOC. Secondly, at higher (>100 mg carbon/L) DOC concentrations the sorption of contaminants was underestimated.

Afterwards, water samples were collected from 15 points within the Baltic Sea in a north-south gradient to examine the spatial differences in DOC characteristics and sorption properties. The DOC samples were analyzed using proton nuclear magnetic resonance and ultraviolet spectroscopy. Results from both techniques indicated that the aromatic nature of the DOC pool increased towards the northern Baltic Sea. This was expected as the freshwater inflow has high significance in controlling the hydrographic conditions in the Bothnian Bay. Sorption of organic contaminants was subsequently measured in the same samples. The results showed decreased sorption from north to south for hydrophobic contaminants such as chlorinated benzenes but for contaminants like tributyl-phosphate no spatial tendencies were observed. The data generated was used to determine molecular descriptors of DOC using linear free energy relationships. The results indicated a higher significance of hydrogen bond donor/acceptor functional groups of the DOC in the south.

Changes in contaminant distribution were simulated in model pelagic ecosystems at possible endpoints predicted by future climate change scenarios. Separate and combined effects of temperature and DOC were studied in mesocosms. The results indicated interesting tendencies. Increased temperature resulted in increased losses in the amounts of organic contaminants. Increased DOC levels promoted sedimentation and sorption of contaminants to particulate matter and biota. Higher amounts of contaminants were retained. The combined effects of the two factors led to an overall decrease in dissolved amounts. Higher losses or increased sedimentation and sorption to particles were also observed depending on contaminant properties.

**Keywords:** climate change, organic contaminants, sorption, dissolved organic carbon, bioavailability.

**Number of pages:** 88 + 5 papers
Bioavailability of Organic Contaminants in a Changing Climate

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Umeå University
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Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
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<tr>
<td>RF</td>
<td>radiative forcing</td>
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<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
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<tr>
<td>AR</td>
<td>Assessment Report</td>
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<tr>
<td>SMHI</td>
<td>Swedish Meteorological and Hydrological Institute</td>
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<tr>
<td>N:P ratio</td>
<td>nitrogen to phosphorus ratio</td>
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<tr>
<td>RCM</td>
<td>regional climate model</td>
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<tr>
<td>SST</td>
<td>sea surface temperature</td>
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<tr>
<td>ADOC</td>
<td>allochthonous dissolved organic carbon</td>
</tr>
<tr>
<td>$K_{OW}$</td>
<td>octanol-water partitioning coefficient</td>
</tr>
<tr>
<td>$K_{AW}$</td>
<td>air-water partitioning coefficient</td>
</tr>
<tr>
<td>H</td>
<td>Henry’s Law constant</td>
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<tr>
<td>DOM</td>
<td>dissolved organic matter</td>
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<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
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<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>TEQ</td>
<td>toxic equivalency factor</td>
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<tr>
<td>Ah receptor</td>
<td>aromatic hydrocarbon receptor</td>
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<tr>
<td>POP</td>
<td>persistent organic pollutant</td>
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<tr>
<td>PAH</td>
<td>polycyclic aromatic hydrocarbon</td>
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<tr>
<td>PCB</td>
<td>polychlorinated biphenyl</td>
</tr>
<tr>
<td>PBDE</td>
<td>polybrominated diphenyl ether</td>
</tr>
<tr>
<td>PeCBz</td>
<td>pentachlorobenzene</td>
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<tr>
<td>HxCBz</td>
<td>hexachlorobenzene</td>
</tr>
<tr>
<td>HCH</td>
<td>hexachlorocyclohexane</td>
</tr>
<tr>
<td>DDT</td>
<td>dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>OP</td>
<td>organophosphate</td>
</tr>
<tr>
<td>TCEP</td>
<td>tris-(2-chloroethyl) phosphate</td>
</tr>
<tr>
<td>TDCIPP</td>
<td>tris-(1,3-dichloroisopropyl) phosphate</td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
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<td>UV</td>
<td>ultraviolet radiation</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>UV-VIS</td>
<td>ultraviolet-visible radiation</td>
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<tr>
<td>SUVA</td>
<td>selective ultraviolet absorption</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectroscopy</td>
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<tr>
<td>FT-ICR</td>
<td>Fourier-transform ion cyclotron resonance</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
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<tr>
<td>Q-TOF</td>
<td>quadrupole time-of-flight</td>
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<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
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<tr>
<td>K&lt;sub&gt;DOC&lt;/sub&gt;</td>
<td>dissolved organic carbon – water distribution coefficient</td>
</tr>
<tr>
<td>K&lt;sub&gt;POC&lt;/sub&gt;</td>
<td>particulate organic carbon – water distribution coefficient</td>
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<tr>
<td>SPME</td>
<td>solid-phase microextraction</td>
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<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
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<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
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<tr>
<td>PS-DVB</td>
<td>polystyrene-divinylbenzene co-polymer</td>
</tr>
<tr>
<td>WBL</td>
<td>water boundary layer</td>
</tr>
<tr>
<td>D</td>
<td>diffusion constant</td>
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<tr>
<td>QSAR</td>
<td>quantitative structure-activity relationship</td>
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<tr>
<td>LSER</td>
<td>linear free energy relationship</td>
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<tr>
<td>SPMD</td>
<td>semi-permeable membrane device</td>
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<tr>
<td>POCIS</td>
<td>polar organic chemical integrative sampler</td>
</tr>
<tr>
<td>PRC</td>
<td>performance reference compound</td>
</tr>
<tr>
<td>RSD%</td>
<td>relative standard deviation (in %)</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half measure</td>
</tr>
<tr>
<td>UHR</td>
<td>ultra high resolution</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>NRFA</td>
<td>Nordic Reference fulvic acid</td>
</tr>
<tr>
<td>UMF</td>
<td>Umeå Marine Sciences Centre</td>
</tr>
<tr>
<td>LLE</td>
<td>Liquid-liquid extraction</td>
</tr>
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List of papers included in the study:

This thesis is based on the following papers referenced in the text by the Roman numerals given in the following list:


II. Ripszam M.; Haglund P.; Automated method for determination of dissolved organic carbon-water distribution constants of structurally diverse pollutants using pre-equilibrium solid-phase microextraction. Environmental Toxicology and Chemistry, in press, 2015, DOI: 10.1002/etc.2805

III. Ripszam M.; Paczkowska J.; Figueira J.; Veenaas C.; Haglund P.; Dissolved organic carbon quality and sorption of organic pollutants in the Baltic Sea in light of future climate change. Accepted to Environmental Science and Technology, DOI: 10.1021/es504437s

IV. Ripszam M.; Gallampois C.M.J.; Berglund Å.; Larsson H.; Andersson A.; Haglund P.; Effects of predicted climatic changes on fates of organic contaminants in brackish water mesocosms Acceptable with minor revision in Science of the Total Environment.

V. Andersson A.; Meier H.E.M.; Ripszam M.; Rowe O.; Wikner J.; Haglund P.; Eilola K.; Legrand C.; Figueroa D.; Paczkowska J.; Lindehoff E.; Tysklind M.; Elmgren R.; Projected future climate change and Baltic Sea ecosystem management. Acceptable with minor revision in AMBIO.
Author’s contributions:

**Paper I**: Recognition of the interference, instrumental work and interpretation. Writing the paper.

**Paper II**: Method development, instrumental and data analysis, writing the paper.

**Paper III**: Planning and execution of sampling, shared instrumental analysis, data analysis and modeling, writing the paper.

**Paper IV**: Shared planning, preparation and execution of the experiment, sample preparation, instrumental and data analysis, writing the paper.

**Paper V**: Experimental data from Paper II, writing the respective section of the paper.
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1. Introduction

Climate change has been shown to be one of the most challenging issues facing humankind in the 21st century. Its main cause is almost certainly the anthropogenic emission of greenhouse gases (CO₂, N₂O, CH₄, etc.). The reported changes in concentrations of these contaminants affect several parameters such as mean temperature, precipitation patterns, wind and jet-stream speeds and directions, and the occurrence of extreme weather events. Such changes induce alterations in the Earth’s hydrosphere, increasing sea surface temperatures, and changing sea surface salinity or mixing depth.

The Baltic Sea is a brackish body of water, which is by definition a mixture of marine and freshwater with a salinity ranging from 0.5 ‰ to 30 ‰. It is a very specific attribute of semi-enclosed seas that their hydrology is regulated by rivers and the narrow straits by which they are connected to saline (30 ‰ – 50 ‰) water masses. The Baltic Sea is very special case because it is divided into three distinct basins (Bothnian Bay, Bothnian Sea and Baltic Proper) that span large temperature, salinity and terrestrial organic carbon gradients.

Regional climate change scenarios predict increased temperatures and precipitation for the Baltic Sea catchment area. These predictions foreshadow increasing temperatures and larger freshwater inputs into the northern parts that will lead to further desalination and higher inputs of terrestrial (allochthonous) organic carbon. These factors all lead to the conclusion that, in the future, the aforementioned gradients may shift towards the south. In fact it has been reported that the “brownification” of the Baltic Sea water has already occurred during recent decades.

The association of organic contaminants to dissolved organic carbon (DOC) has been shown to have an immense impact on their bioavailability. These organic contaminants may enter the food-web at a microbial level by
either direct uptake in phytoplankton-based (autotrophic) food-webs, or through sorption to DOC, which serves as a food source in bacteria-based food-webs.

This PhD project had two main focuses. The first was to investigate the differences in DOC quality at various points of the Baltic Sea and to determine the extent to which DOC influences the partitioning of organic contaminants. In this part of the project we used molecular descriptors to explain differences in partitioning and sorption process at a molecular level.

The other main focus was to investigate how different environmental drivers (temperature and DOC content) might affect the distribution of organic contaminants by the end of the 21st century at various endpoints predicted by regional climate change scenarios in simulated Baltic Sea ecosystems. This included a large-scale mesocosm experiment.
2. Climate change

2.1. Global effects and scenarios of climate change

It is well proven from multiple independent datasets dating back to the late 19th century, that the surface temperature of the Earth has been undergoing a steadily increasing trend [1]. It is undeniable that the increase in combined land and ocean surface temperatures ranged from 0.65 °C to 1.08 °C in the period from 1880 to 2013, and has increased at an especially high rate in the last four decades (Figure 1) [2]. Changes in water cycling have also caused shifts in global precipitation patterns with increasing trends in the temperate, subarctic and arctic climates in the northern hemisphere.

Figure 1: Decadal combined ocean and land surface temperatures from 1850 to 2012 relative to the average temperature in the 1961 - 1990 period. Figure approximately reconstructed based on the IPCC fifth assessment report [2].

Indicators of climate change include such variables as rising sea level, decreasing pH and increasing dissolved CO₂ concentrations in ocean waters, diminishing sea-ice coverage, and increasing loss of glacial ice. Radiative
forcing \((RF)\) has been established as a factor that quantifies the extent to which each climate change driving force contributes to the energy budget of the Earth. For all the “well mixed” greenhouse gases (\(CO_2\), \(CH_4\), halo-carbons and \(N_2O\)) we are more than 90% certain that their contribution to the \(RF\) can be seen to have increased from 1750 to 2011. It has also been pointed out with a high level of confidence that the anthropogenic contribution to the increased \(RF\), has itself increased within the last half century [3].

Climate change scenarios that have been suggested in each IPCC assessment report (AR) have so far been based on actual results concerning greenhouse gas emissions and various climate change indicators. In \(AR5\), four new emission scenarios were suggested, but in the present study, scenarios suggested in \(AR4\) will also be mentioned, simply because all the dynamic downscaled regional models generated for the Baltic Sea area used global predictions of the \(AR4\) scenarios [4]. In the \(AR4\) report, three scenarios were suggested for greenhouse gas emissions: A2 being the least optimistic, B1 as the most optimistic, and A1B falling in between. As the details are available in \(AR4\) they will not be discussed any further in this thesis.

Because of the large ranges (both in time and space) over which environmental processes operate, the uncertainties of the predictive model scenarios are quite large and increase as time progresses. So far, temperatures follow the projections suggested by the first assessment report in 1990. The recent \(CO_2\) concentrations follow the \(A2\) scenario, and methane is closest to \(B1\) (the most optimistic). It will be very important to utilize the scenarios presented in \(AR5\) to further refine future predictive models in order to generate more accurate predictions of what might occur in years to come.

2.2. The Baltic Sea environment

The Baltic Sea is the second largest brackish sea on Earth (after the Black Sea). Its salinity is controlled by the annual inflow of freshwater and
periodic water exchange with the North Sea. It drains an area which is home to approximately 90 million people of 14 countries [5]. The Baltic Sea itself cannot be regarded as a single mass of water. There are significant north-south gradients in salinity, temperature and productivity. Based on this we can distinguish three basins (Figure 2): the Bothnian Bay, the Bothnian Sea and the Baltic Proper.

![Map of Baltic Sea basins and gradients](image)

**Figure 2:** The main Baltic Sea basins and the existing north-south gradients. Color coding from left to right: Temperature: blue (cold) and red (warm), ADOC: brown (more ADOC) white (less ADOC), salinity: green (more) and yellow (less), ecosystem production: green (higher) and brown (lower).
The three basins show major differences in the extent of freshwater influx. The inflow of freshwater is ~3100 m$^3$ s$^{-1}$ in the Bothnian Bay, ~2850 m$^3$ s$^{-1}$ in the Bothnian Sea and ~3900 m$^3$ s$^{-1}$ in the Baltic Proper. Data were taken using the monthly averages for 2014 from all drainage areas provided by Swedish Meteorological and Hydrological Institute (SMHI) [6]. At first glance these values might appear to be quite even, but the impact of freshwater influx needs to be re-evaluated if the average volumes of these basins are to be taken into consideration. The volume of the Baltic Proper takes up 51% of the whole Baltic Sea with almost 12500 km$^3$ whereas the volumes of the Bothnian Sea and the Bothnian Bay are approximately 4300 km$^3$ and 1500 km$^3$, respectively [7]. If we normalize the annual mean influxes to the unit volume (1 km$^3$) of each basin we get a more descriptive picture. These values are ~0.3 m$^3$ s$^{-1}$ for the Baltic Proper, ~0.6 m$^3$ s$^{-1}$ and ~2.1 m$^3$ s$^{-1}$ for the Bothnian Sea and Bothnian Bay, respectively. Overall, the northern basins of the Baltic Sea are affected by the freshwater influxes 3 - 7 times more than the southern basin. This process contributes significantly to the salinity gradient which ranges from ~3 ‰ in the Bothnian Bay to ~8 ‰ in the southern Baltic Proper. Periodic saline (> 25 ‰) water inflow events from the North Sea through the Danish sills from Kattegat counteract the desalination of the Baltic Sea [8].

Vertical stratification also exists within the Baltic Sea. This is mainly driven by water density gradients caused by differences in temperature, salinity and mixing depth. The thin transition zones are called “clines”, which are the layers where the gradient of a certain water property is the steepest. Two of the most commonly known layers are the thermocline with a steep temperature gradient, and the halocline with a steep salinity gradient. Even though there are strong seasonal variations in the position of these “clines”, the thermocline and halocline are normally located 30 m – 40 m and 60 m – 80 m beneath the surface, respectively [8].
A large inflow of freshwater is accompanied by a large amount of organic carbon of terrestrial origin. A fraction of this organic carbon flocculates almost immediately upon contact with the saline water [9]. This flocculated particulate organic matter sinks to the benthos close to the coastlines and is then mineralized – a process which consumes dissolved oxygen – thus being one of the main drivers behind the hypoxic conditions throughout the Baltic Sea. The inflowing freshwater is also rich in nutrients (bioavailable nitrogen and phosphorus compounds) into the Baltic Sea. The high N:P ratio in the north has been widely reported in the literature [10]; it causes eutrophication throughout the Baltic Sea which also leads to anoxic conditions due to high consumption of dissolved oxygen.

The extent of the dissolved fraction of organic carbon is mainly defined by its solubility, which is mostly controlled by parameters like pH and salinity (ionic strength) [11-14]. Even though there are vast differences in the inflow of organic matter, there is no such difference in DOC concentrations throughout the pelagic Baltic Sea. Nevertheless, differences in bulk chemical characteristics of the DOC mixture can be suspected whether one merely looks at the color of the water at different latitudes, or whether one examines the residence time [15, 16] of the allochthonous - to - autochthonous ratios [16] of DOC (terrestrial or marine origin).

2.3. Predicted future changes of the Baltic Sea environment

Climate change will have different effects at different regional scales. These effects will not necessarily be the same everywhere on the planet. Regional downscaling methods were therefore developed in order to predict the local effects of climate change on the Baltic Sea. These are called regional climate model (RCM) simulations. Several studies use different variations of this method such as high-resolution, dynamic, and transient RCM simulations [17-
To estimate climate change effects in the Baltic Sea area, the two most realistic scenarios were applied (A1B and B1). Different greenhouse gas emission scenarios have been proposed by the IPCC AR4 [4].

The Baltic Sea receives a continuous inflow of pollutants, nutrients and organic matter from its surrounding region and its ecosystem is also significantly affected by overfishing [16, 22]. These circumstances could cause eutrophication and several other negative effects on the Baltic Sea ecosystem. Future climate change in the Baltic Sea area will have primary and secondary effects on the ecosystem. On a local level, the RCM simulations (for the 1960 – 2100 intervals) predict an increase in air and sea surface temperatures by up to 2 °C - 3 °C until 2100 in every season of the year, and an increase in the annual precipitation. These primary effects will result in various secondary effects.

Increased sea surface temperature (SST) and freshwater inflow are two of the most pronounced events to take place that can lead to decreasing salinity of the Baltic Sea. Acidification will probably increase due to a higher concentration of atmospheric CO$_2$ and decreased coverage by winter ice. The amount of sea-ice at the end of the 21st century is expected to be around one-third of the current state. Changes in salinity and increased wind speeds will result in an altered vertical stratification that will probably lead to changes in temperature, salinity, hypoxia, etc. that could push organisms closer to their tolerance limits.

As a result of higher precipitation the inflow of terrestrially derived organic substances (allochthonous dissolved organic carbon – ADOC) will increase along with the nutrient (N, P) inflow, which partly results from decreased permafrost. The inflow of ADOC may result in increased light attenuation and, most importantly for us, an increase in pollutant load and potential changes in pollutant bioavailability. The increased amount of N and P inflow will result in increased eutrophication.
From an ecological standpoint, the effect of increased ADOC inflow reportedly promotes a shift from a phytoplankton based towards a more bacteria based food-web as a consequence of larger organic carbon load and decreased photosynthetically active radiation due to light attenuation [10, 23]. Phytoplankton based food webs are mostly driven by nutrient availability (dissolved nitrogen and phosphorus) and light and is mostly autotrophic with lower oxygen consumption, higher biomass and chlorophyll concentration. Bacteria based food-webs are less productive, less dependent on light, more oxygen consuming, and based on both nutrients and organic carbon. Bacteria based food webs also may include grazer communities (ciliates, flagellates) that introduce an extra trophic level to the food-chain.

These simultaneous effects will generate changes in food-web dynamics and structure. Because little is known at present concerning how these drivers collectively act on the ecological system, it is essential to take these effects into account in connection with climate change.
3. Pollutants in a changing climate

3.1. Global pollutant cycling

Climate change has been shown to have a large effect on the redistribution of organic contaminants on Earth as their reactivity, bioaccumulation, sorption, transport and partitioning processes all depend on temperature [24-29]. Changes in precipitation patterns will lead to differences in contaminant deposition. In the case of rain, the water droplets provide an air-water interface across which compounds partition. Increased snowfall provides even more of a material that has a very high effective surface area (up to 1 m² g⁻¹) onto which very hydrophobic contaminants or particulate contaminants suspended in the air can be adsorbed or bound to, and which are then deposited on land or water surfaces [24].

MacDonald has described the environmental concentration of organic contaminants by two main processes. One is called “solvent switching” whereby compounds are transferred from one phase to another driven by equilibrium partitioning constants (\(K_{OW}\), \(K_{AW}\), \(H\), etc.). These parameters determine the general behavior of the contaminants in the various environmental compartments. This is exemplified by the long-range transport of \(\alpha\)- and \(\beta\)-HCHs. Because the \(\beta\)-HCH conformer partitions 20-fold more into water than \(\alpha\)-HCH, it cannot be found beneath the arctic ice [24]. This concept has also been referred to as “global fractionation” or “global chromatograph” model where the environmental compartments are compared to a chromatographic separation. The stationary phase is water, soil and vegetation and the moving phase is air. The transport of contaminants depends on the partitioning constants [24, 30, 31]. The more volatile contaminants will travel farther and faster.

The other process is called “solvent depletion” whereby the contaminants concentrate selectively by the investment of energy. Bioaccumulation is such a process in which the lipids within an organism act as the “solvent”. As nutrients
move up the food chain, the various organisms metabolize lipids while accumulating the whole supply of persistent contaminants from their food and the media they live in (air, water). This results in much higher contaminant concentrations in fatty tissues than the thermodynamic equilibrium partitioning constants would otherwise allow. Several other processes, such as snow sintering and melting, and starvation, lead to higher contaminant concentrations in freshwater and in the bloodstream of animals, respectively. Climate change is likely to affect all of these biogeochemical processes, whether from an increase in equilibrium partitioning constants as an effect of temperature, or increased deposition by precipitation, the introduction of additional trophic levels in an ecosystem, or the limitation of available prey. All these climate change induced alterations are likely to affect the global distribution of contaminants.

Another interesting result of climate change is the appearance of secondary sources of persistent organic pollutants (POPs) [32]. Although these chemicals remain in the environment as a legacy of their use by industry from the middle of the 20th century up to their being banned in the 1970s, their emission has since been greatly reduced. However, as a result of long-range transport, these pollutants have been deposited and “archived” in places such as the Arctic/Antarctic or in high altitude glaciers far from their sources of emission. With the remission of ice-caps and the elevation of snowlines during summer time as a result of climate change, these “stored” POPs can re-enter the global cycling of organic contaminants. Other effects of climate change such as an increase in the frequency of extreme events, such as the massive flood caused by Hurricane Katrina, will lead to the further redistribution of organic contaminants [33, 34]. Secondary effects of climate change, such as shifts in agriculture and related issues, could lead to higher survival rates of pests due to milder winters and the potential development of resistance against pesticides, which may also have a significant influence on contaminant loads [35].
3.2. Pollutant cycling and effects in the Baltic Sea

It is very challenging to sum up the climate induced processes that could affect pollutant cycling within the Baltic Sea. The projected climate change induced effects on the Baltic Sea are summarized in Figure 3. Regional climate change scenarios predict increased temperatures (of 2 °C – 4 °C), increased precipitation during winters, warmer and drier summers, and more extreme weather events and higher wind speeds in the catchment area of the Baltic Sea [17, 21, 27, 36].

![Figure 3: Schematic summary of climate change induced effects on the biogeochemical processes and resulting alterations in organic contaminant cycling in the Baltic Sea.](image)

Increased precipitation and decreased permafrost are likely to induce a larger washout of terrestrial (allochthonous) organic carbon into the Baltic Sea. These main effects will have strong implications on other processes that heavily influence the cycling and redistribution of contaminants in all compartments.
This topic is discussed below, starting with processes taking place in the atmosphere, then dealing with the hydrosphere, and continuing with the partitioning of contaminants into sediments where they will be “stored” and later mineralized.

Increased precipitation during winter will promote the deposition of airborne contaminants out of the atmosphere. It will also make available a greater snow surface area for the adsorption of contaminants, and more rain droplets for partitioning contaminants. Higher average temperatures will lead to decreased permafrost that will result in increased mobilization and volatilization of contaminants through both soil and vegetation. This can lead to the redistribution of contaminants through increased volatilization during the warmer and drier summers and through washout of organic matter with which contaminants are associated.

Alterations in the water body are mainly governed by increased temperature, precipitation and wind speed. The consequent changes include: (i) increased sea surface temperatures [21]; (ii) decreased surface salinity [17]; (iii) rising sea-levels and decreased ice cover; (iv) changes caused by the higher inflow of organic carbon such as acidification, hypoxia and light attenuation; (v) altered species distribution [37]; and (vi) eutrophication, which has already been reported to be a serious issue of significant concern in the Baltic Sea due to the inflow of nutrient-rich (especially in nitrogen) freshwater [5, 38]. Consequent changes in pollutant cycling and redistribution are expected to follow. Some results of increased water temperature include increased volatilization, increased degradation rates, and increased enzyme activity. Higher organic carbon inflow may lead to increased adsorption to particles and partitioning to DOC so decreasing the bioavailability of contaminants via direct uptake. “Brownification” due to the increased influx of colored DOM (dissolved organic matter) produces reduced photo-degradation rates. The shift towards bacteria based food-webs and the introduction of extra trophic levels can result in altered
biomagnification routes and therefore, changed bioaccumulation factors. Changes in species distribution and migration as a consequence of shifts in environmental conditions might also affect the cycling of pollutants through altered transport and bioaccumulation pathways.

The partitioning of organic contaminants to sediments is likely to be affected mostly by processes such as wind speed and the related occurrence of extreme weather events, as well as certain anthropogenic activities like fishing and dredging. The resulting re-suspension of contaminants by all the aforementioned processes and activities are counteracted by increased sedimentation due to a larger DOC inflow. Shifts in benthic organism populations such as the well-documented ongoing invasion of *Marenzelleria* and the decreased population of *Monoporeia affinis* can also change the extent of bioturbation; *Marenzelleria* has been shown to be an efficient agent in the bioturbation and bioaccumulation of POPs [39, 40].
4. Organic contaminants studied

For Paper I we used the 16 priority polycyclic aromatic hydrocarbons (PAHs) that can be found on the US EPA priority pollutant list [41]. The organic contaminants that we used for the studies described in Paper II – Paper V were based on the list of hazardous materials presented by the European Commission in 2008 [42]. The list of these substances is presented in Table 1 and their structures are given in Figure 4. The organic compounds that were selected for testing were chosen not only in order to cover different groups of organic contaminant/pollutant classes, but also to include examples with clear structural elements (hence the inclusion of anilines and phenols with different degrees of bromination). Because DOC sorption processes were the focus of most studies, the selected compounds were relatively hydrophobic, with octanol-water partitioning constants (log \(K_{OW}\)) in the range \(\log K_{OW}\) 1.5 – 6.5.

PAHs are mainly formed during incomplete combustion of fossil fuels and are ubiquitous in all compartments of the environment [43, 44]. They have low water solubility and are lipophilic. PAHs have been shown to give rise to a number of adverse effects; in particular, some higher molecular weight congeners have highly carcinogenic effects both in animals and humans. The International Agency for Research on Cancer - IARC classified a number of PAHs to Group 1 (carcinogenic to humans) to Group 2B (possibly carcinogenic to humans). [44-46]. They have been shown to be susceptible to photolytic and enzymatic transformations and they react with atmospheric gases. The products of these reactions are often very carcinogenic and mutagenic as well [47, 48].
Table 1: List of investigated contaminants discussed in Papers II-V.

<table>
<thead>
<tr>
<th>POP convention</th>
<th>Water framework directive</th>
</tr>
</thead>
<tbody>
<tr>
<td>pentachlorobenzene</td>
<td>biphenyl</td>
</tr>
<tr>
<td>HCB</td>
<td>naphtalene</td>
</tr>
<tr>
<td>α-HCH</td>
<td>fluorene</td>
</tr>
<tr>
<td>β-HCH</td>
<td>phenanthrene</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>anthracene</td>
</tr>
<tr>
<td>δ-HCH</td>
<td>hexachlorobutadiene</td>
</tr>
<tr>
<td>endosulfan I</td>
<td>chlorpyrifos</td>
</tr>
<tr>
<td>endosulfan II</td>
<td>trans-chlorfenvinfos</td>
</tr>
<tr>
<td>PCB-2</td>
<td>alachlor</td>
</tr>
<tr>
<td>PCB-11</td>
<td>atrazine</td>
</tr>
<tr>
<td>PCB-28</td>
<td>trifluralin</td>
</tr>
<tr>
<td>mitotane</td>
<td>octylphenol</td>
</tr>
<tr>
<td>Emerging contaminants</td>
<td>chlorothalonil</td>
</tr>
<tr>
<td>tributyl phosphate</td>
<td>chlorothal dimethyl</td>
</tr>
<tr>
<td>TDCIPP</td>
<td>chlorpyrifos methyl</td>
</tr>
<tr>
<td>TCEP</td>
<td>pendimethalin</td>
</tr>
<tr>
<td>triphenyl phosphate</td>
<td>picoxystrobin</td>
</tr>
<tr>
<td>dibutyl phthalate</td>
<td>diflufenican</td>
</tr>
<tr>
<td>4-bromophenol</td>
<td>diazinon</td>
</tr>
<tr>
<td>2,4-dibromophenol</td>
<td></td>
</tr>
<tr>
<td>2,4,6-tribromophenol</td>
<td></td>
</tr>
<tr>
<td>4-bromoaniline</td>
<td></td>
</tr>
<tr>
<td>2,4-dibromoaniline</td>
<td></td>
</tr>
<tr>
<td>2,4,6-tribromoaniline</td>
<td></td>
</tr>
</tbody>
</table>
### Chemical structures of the studied compounds

<table>
<thead>
<tr>
<th>Pentachlorobenzene</th>
<th>Hexachlorobenzene</th>
<th>Hexachlorocyclohexanes</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Pentachlorobenzene" /></td>
<td><img src="image" alt="Hexachlorobenzene" /></td>
<td><img src="image" alt="Hexachlorocyclohexanes" /></td>
</tr>
<tr>
<td>Endosulfans</td>
<td>PCBs (and biphenyl)</td>
<td>Mitotane</td>
</tr>
<tr>
<td><img src="image" alt="Endosulfans" /></td>
<td><img src="image" alt="PCBs (and biphenyl)" /></td>
<td><img src="image" alt="Mitotane" /></td>
</tr>
<tr>
<td>Tributyl phosphate</td>
<td>TCDIPP</td>
<td>TCEP</td>
</tr>
<tr>
<td><img src="image" alt="Tributyl phosphate" /></td>
<td><img src="image" alt="TCDIPP" /></td>
<td><img src="image" alt="TCEP" /></td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>Dibutyl phthalate</td>
<td>Bromophenols (e.g. 2,4-di-)</td>
</tr>
<tr>
<td><img src="image" alt="Triphenyl phosphate" /></td>
<td><img src="image" alt="Dibutyl phthalate" /></td>
<td><img src="image" alt="Bromophenols" /></td>
</tr>
<tr>
<td>Bromoanilines (e.g. 4-mono-)</td>
<td>Naphthalene</td>
<td>Fluorene</td>
</tr>
<tr>
<td><img src="image" alt="Bromoanilines" /></td>
<td><img src="image" alt="Naphthalene" /></td>
<td><img src="image" alt="Fluorene" /></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>Anthracene</td>
<td>Hexachlorobutadiene</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><img src="image1.png" alt="Phenanthrene structure" /></td>
<td><img src="image2.png" alt="Anthracene structure" /></td>
<td><img src="image3.png" alt="Hexachlorobutadiene structure" /></td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Chlorpyrifos*</td>
<td>Chlorfenvinfos</td>
</tr>
<tr>
<td><img src="image4.png" alt="Chlorothalonil structure" /></td>
<td><img src="image5.png" alt="Chlorpyrifos structure" /></td>
<td><img src="image6.png" alt="Chlorfenvinfos structure" /></td>
</tr>
<tr>
<td>Alachlor</td>
<td>Atrazine</td>
<td>Trifluralin</td>
</tr>
<tr>
<td><img src="image7.png" alt="Alachlor structure" /></td>
<td><img src="image8.png" alt="Atrazine structure" /></td>
<td><img src="image9.png" alt="Trifluralin structure" /></td>
</tr>
<tr>
<td>Octylphenol</td>
<td>Chlorothal dimethyl</td>
<td>Pendimethalin</td>
</tr>
<tr>
<td><img src="image10.png" alt="Octylphenol structure" /></td>
<td><img src="image11.png" alt="Chlorothal dimethyl structure" /></td>
<td><img src="image12.png" alt="Pendimethalin structure" /></td>
</tr>
<tr>
<td>Picoxystrobin</td>
<td>Diflufenican</td>
<td>Diazinon</td>
</tr>
<tr>
<td><img src="image13.png" alt="Picoxystrobin structure" /></td>
<td><img src="image14.png" alt="Diflufenican structure" /></td>
<td><img src="image15.png" alt="Diazinon structure" /></td>
</tr>
</tbody>
</table>

Figure 4: Chemical structures of the compounds of interest throughout *Papers II - V*. All structures were drawn using Marvin (ChemAxon).
* chlorpyrifos if $R = -\text{C}_2\text{H}_5$, chlorpyrifos dimethyl if $R = -\text{CH}_3$
Polychlorinated biphenyls (PCBs) are not naturally occurring organic pollutants. They consist of a biphenyl backbone structure with different levels of chlorination (1 – 10), which makes 209 congeners theoretically possible. PCBs are fire resistant with low electrical and high thermal conductivity, extremely good thermal stability, and low reactivity. They are very lipophilic, and extremely persistent. PCBs had been used as dielectric and heat exchange fluids in electronic equipment until the detection of their abundance in the environment in 1966. Since then, most governments have banned their commercial use [49-51]. Toxicity of PCBs is measured by the TEQ concept used for dioxin-like compounds. PCBs have been shown to have effects that may be acute but which are mostly chronic (through binding to the cytosolic Ah receptors), as well as hepatocarcinogenic and some mutagenic effects [51-55]. The IARC classifies 12 PCB congeners as Group 1 [56].

Hexachlorocyclohexanes (HCHs), penta- and hexachlorobenzene (PeCBz, HxCBz) have been commercially used as insecticides and fungicides. Their halogenated aromatic structures give them similar levels of inertness and persistence as PCBs. PeCBz and HxCBz are lipophilic and accumulate, whereas HCHs are less lipophilic and have higher water solubility than HxCBz. Adverse effects of these pollutants have been reported widely in the literature. They are also classified as carcinogenic and endocrine disruptive compounds by both the IARC and the US EPA [55, 57-59].

Organophosphates (OPs) are one class of emerging contaminants that are used as flame-retardants in polymers, textiles and electronic equipment. With the intention to replace the very toxic, bioaccumulative and persistent brominated flame-retardants, the use of these compounds has increased in the last decade [60, 61]. The non-chlorinated OPs are also used as plasticizers [61]. These compounds are less lipophilic, persistent and bioaccumulative than PCBs or chlorobenzenes. Most OPs can be found in indoor air, surface water and sediments. Effects of OPs are generally carcinogenicity and neurotoxicity
TCEP has reportedly mutagenic effects and has recently been added to the EU candidate list [62] and several other OP flame-retardants have recently been included in the EU toys directive [63].

There are a number of pesticides included in this thesis that belong to the organochlorine pesticide category (endosulfans, chlorothalonil, and chlorthal dimethyl) that are very persistent and bioaccumulative. The remaining compounds are organophosphate pesticides (chlorpyrifos, diazinon and chlorfenvinfos) that are less persistent, and others, such as diflufenican, picoxystrobin, atrazine and pendimethalin. Many of these pesticides are currently used worldwide. The harmful nature of these compounds spans a wide range of responses in different organisms that are often carcinogenic and neurotoxic [55, 64-68].
5. Dissolved organic matter and dissolved organic carbon

Dissolved organic matter (DOM) is technically defined as the fraction of organic carbon that passes through a filter with a pore size of 0.2 µm – 0.7 µm, although different studies state other pore sizes for this threshold. In this thesis “dissolved” is being referred to as the most widely used sub - 0.45 µm originating from the 1950s when cellulose acetate membranes were first used [69]. This size fraction includes dissolved material and a fraction of colloidal organic molecules [70]. The collective mixture of DOM cannot be quantified. Its main “building block”, dissolved organic carbon (DOC) is instead used to describe the extent of DOM in natural waters [71]. DOC is the largest reservoir of reactive organic carbon on Earth and it is viewed as a short term “sink” for atmospheric CO₂ [72]. The total amount of DOC that resides in the world’s oceans is estimated to be approximately 700 Gt, which is comparable to the inorganic carbon that can be found in the atmosphere [73]. The net oxidation of 1 % of this organic carbon mixture has been proved to be enough to generate a higher CO₂ flux than that emitted annually from the oxidation of carbon originating from fossil fuels. Research on DOC has become especially popular in recent decades because of its connection to the global carbon cycle, atmospheric CO₂, and climate change [72].

DOC is a mixture of a wide range of compounds that can be found in all natural waters. This organic mixture can be produced in situ by organisms or can be a variety of degradation products of various origin. Human activities also contribute to the DOC pool, whether from organic compounds leaching out into the soil, or their dispersion into the air from urban areas. One can consider humic and fulvic acids as the two most persistent building blocks of DOC [74]. They can be distinguished by their solubility under highly acidic (pH = 2) conditions. The insoluble fraction is the humic acid fraction while the fulvic acid fraction is soluble [75]. Its more reactive components are oligomeric carbohydrates, lipids, peptides and lignin (Figure 5).
John I. Hedges introduced two different methods for both the qualitative and quantitative examination of DOC [72]. One is a holistic approach, which basically involves the investigation of DOC as a bulk mixture and examining its collective behavior. This is a valuable approach if we are trying to describe its parameters in situ, but it yields only limited information on the chemical structure of DOC constituents. The “reductionist” approach, on the other hand, focuses on certain constituents and isolated DOC fractions and is based on observations that are used by extrapolation to infer properties of the bulk mixture. In the studies presented in the thesis, both approaches are utilized and we attempt to make connections between collective behavior and certain molecular characteristics.

There are two main methods used to determine the total amounts of DOC. One is to measure the amount of total organic carbon after filtration using wet or catalytic oxidation of DOC and then to measure a certain property related to the released amount of CO₂. This can be performed by colorimetric or other types of detection method [76, 77]. Nowadays, the most widely used routine
quantitative analysis of DOC is done by high temperature combustion and subsequent measurement of CO₂ using infrared spectrometry. This technique was used to determine DOC concentrations during this study.

5.1. Methods to characterize DOC

The characterization of DOC can be performed in situ and also after separation and isolation. Most methods have limitations in that they can only be applied after isolation or extraction. The two most widely used isolation methods to prepare DOC for such experiments are solid phase extraction (SPE) and ultrafiltration [15, 78, 79]. The disadvantages of these methods are briefly: (i) isolation using SPE results in losses of the more hydrophilic fraction (aromatic and hydrophobic enrichment), (ii) ultrafiltration leads to a loss in the low molecular weight fraction. However, the combination of ultrafiltration and SPE could result in a very efficient recovery [80].

Fluorescence spectroscopy is a good example for the in situ characterization of DOC since it is a technique sensitive enough to be applicable even for marine water samples. Many different applications of this technique have been reported. It is very useful for assessing DOCs of diverse origin, as well as the detection of certain protein-like substructures containing fluorescent amino acid side chains [81, 82]. Its limitation is its specificity towards constituents that contain fluorophores. A more non-specific optical technique is ultraviolet (UV) spectroscopy. Several absorption bands of DOC have been shown to correlate with certain bulk properties. For instance, a specific UV absorption at 254 nm (\(SUVA_{245}\)) is widely used as a proxy parameter to describe the bulk aromaticity of DOC [83, 84] (Figure 6). The ratio of absorbance measured at 465 nm and 665 nm (\(E_{465}/E_{665}\)) has been shown to correlate with molecular size [85-87]. These techniques are useful but they are unable to provide detailed molecular information.
Nuclear magnetic resonance (NMR) spectroscopy is often used to gain more information about the carbon quality and the carbon “backbone” structure of DOC. For these types of measurements an additional concentration step is required due to detectability issues. This is true for proton (\(^1\text{H}\)) NMR and especially for carbon (\(^{13}\text{C}\)) NMR which can only be performed using magic angle solid-state \(^{13}\text{C}\) NMR. There are several studies that have used these techniques [15, 83, 85, 88]. Carbon and hydrogen atoms of different chemical environments (e.g.: aliphatic, alkene or aromatic) can be determined using the chemical shift values. Peaks from the \(^1\text{H}-\text{NMR}\) spectra can be integrated and quantified directly and the ratios of these peaks can be utilized to gain structural information.

Mass spectrometry has been used to characterize DOC with or without liquid chromatography. The use of ultra-high resolution mass spectrometry (UHR-MS) such as FT-ICR (Fourier transform ion cyclotron resonance) became the most efficient tool for analyzing DOC because of its extreme resolution, which allows the determination of exact elemental compositions of the numerous constituents in the DOC aggregates [89-91]. Lower resolution tandem mass spectrometry can be used to generate complementary structural information about such DOC constituents. Triple quadrupole, multiple tandem MS ion trap, and quadrupole-time-of-flight (Q-TOF) MS have all been used for this purpose [74, 92-94]. Gas chromatographic (GC) or liquid chromatographic (LC) separation of DOC, or even humic and fulvic acids usually result in overlapping peaks, and peak shapes which are not well defined. Tandem MS measurements give valuable information about the molecular structure of certain ionizable DOC constituents. For example, the number of functional groups can be deduced from m/z differences in the product ion mass spectra. A loss of 18 m/z and 44 m/z correspond to a loss of water and CO\(_2\), respectively [92, 94]. By using high-resolution MS the elemental composition of these product ions can be further clarified [74].
There are several other methods worth mentioning that can also be used to investigate different parameters of DOC. Such techniques include infrared spectroscopy (functional groups), size exclusion chromatography (UV or MS detection – molecular size), pyrolysis GC-MS, elemental analysis for composition, protein and carbohydrate analysis, δ$^{13}$C for age and origin determination, and $^{14}$C analysis for age determination [15, 16, 85, 95-98].

5.2. Role of DOC in pollutant cycling

The presence of DOC in natural waters has important roles in slowing volatilization, decreasing sediment partitioning, and controlling the solubility, bioavailability, bioaccumulation, toxicity, transformation, reactivity and environmental fate of organic contaminants [99-104]. The sorption mechanisms of contaminants can be quantified by the DOC–water partitioning coefficient ($K_{DOC}$ or log $K_{DOC}$). In natural waters the equilibrium between organic matter and pollutants is a heterogeneous process [101]. The pollutants show significant sorption to the surface of particulate organic matter as well as sorption to DOC in the aqueous phase (Figure 6).

The first question to occur in connection with the sorption of pollutants to DOC is whether the sorption can be described as a one-phase association or a partition process between two different phases (water and DOC). The sorption of contaminants to DOC from a macroscopic point of view can be described by two different considerations. One way is to regard the sorption as a one-phase association between the pollutant and the DOC. In this case a linear relationship is assumed between the free and the bound concentrations of the contaminant [103].
The other way is to consider the water and DOC as a specific second phase in the water. This consideration expects there to be nonlinear relationships between free and bound concentrations. In practice observed isotherms are mostly linear, but when the concentration of the pollutant increases or the binding sites within DOC are limited or competitive processes occur the isotherm can become nonlinear (Langmuir-like) \[103\]. Reportedly, the former process is assumed to take place at ideally dilute contaminant concentrations. In reality, it is easier to depict the partitioning as a mixture of homogeneous and heterogeneous processes. Homogeneous molecular association of contaminants to DOC is likely to happen in case of “truly” dissolved DOC constituents but the sub - 0.45 μm size fraction of natural waters contains colloids which can be perceived as a separate phase \[70\].

Binding affinities of contaminants to DOC can be described as a molecular level process. The structural elements and molecular size of DOC
can significantly affect the binding of different organic contaminants. This binding process was first thought to be unspecific and mostly driven by the hydrophobicity of a pollutant, and so mainly described by the $K_{OW}$ values. On the other hand, studies have shown that, depending on well-defined molecular interactions like aromaticity and steric hindrance, specific contaminants can show unusually high $K_{DOC}$ values [105-107]. A good example is the binding of pyrene to fulvic acids with high (> 20 %) aromaticity [106]. The example of ortho- and non-ortho-substituted PCBs reflects the importance of steric specificity of the sorption to DOC [102]. Furthermore, other examples demonstrate that there is a stoichiometric specificity of sorption processes: for example atrazine and 2,4-dichlorophenoxyacetic acid exhibit a 2:1 pollutant per DOC molecule ratio, whereas for lindane a 1:1 ratio has been measured [105, 107].

However, in soils and sediments DOC is considered to be a polyelectrolyte as its constituent compounds are mostly negatively charged because of the abundant carboxylic and phenolic functional groups; they thus form dielectric layers (a Stern layer to cancel the negative charge of the DOC and a subsequent diffuse negative layer of anions). In conclusion, we might say that the sorption of pollutants to DOC in water environments is driven by molecular factors such as aromaticity or steric hindrance, but that other factors such as varying concentrations of a contaminant and DOC can cause differences in the sorption process [103, 108].
6. Methods to measure $K_{DOC}$ values

In order to determine $K_{DOC}$ we have to know the ratio of a pollutant sorbed to the DOC molecules and its concentration in the freely dissolved phase. In many cases the measurement of the DOC-bound fraction of contaminants can be challenging. Instead, most of these methods include a measurement of the freely dissolved contaminant fraction ($C_{free, smp}$) for a sample that contains DOC and a reference sample ($C_{free, ref}$) with the same conditions but without DOC.

$$K_{DOC} = \frac{C_{DOC-bound}}{C_{free}}$$

$$K_{DOC} = \frac{(C_{free,ref} - C_{free,spm})}{C_{free,spm}}$$

One question about these analytical techniques concerns how they might affect the distribution process. Some methods may lead to significant disruption in the partitioning, while some can cause less bias. For example Burkhard states in his landmark study that sparging, fluorescence quenching, solid phase microextraction (SPME) and equilibrium dialysis causes the least perturbation of the partition process [109]. Nevertheless, equilibrium dialysis, reversed phase and solubility enhancement techniques have limitations in that they can underestimate coefficients at increasing concentration values of DOC [108]. Underestimation of $K_{DOC}$ values might also occur while using pre-equilibrium SPME because of altered uptake kinetics caused by the high DOC concentration.

6.1. Non-invasive methods for $K_{DOC}$ determination

Fluorescence quenching can be used to determine the free aqueous concentration of pollutants that contain a fluorophore (mainly PAHs with few aromatic rings). The limited specificity is one of the limiting factors of this
There are three types of fluorescence quenching: static, dynamic and apparent. Static quenching occurs when the free fluorescent solute forms a DOC-complex, which has an absorption spectra overlap with the fluorophore and thus absorbs the emitted fluorescence. Dynamic quenching involves fluorescence resonance energy transfer (FRET) when the fluorophore (contaminant) gets close enough to the quencher (DOC molecule). Apparent quenching happens when the fluorescence intensity decreases because of the optical density (high concentration of chromophores) of the solution. Its biggest advantage is that it is a completely non-invasive technique. On the other hand, its use is limited for compounds that are fluorescent (mostly used for PAHs) and which have high enough solubility in water for detection [110-112].

The increased solubility of hydrophobic organic contaminants by the presence of DOC is measured by the apparent solubility technique. The solubility of a contaminant measured at various DOC concentrations yields a linear regression from which $K_{DOC}$ can be calculated [103, 113-115]. Sorption studies using $^{14}$C labeled contaminants are also used to determine $K_{DOC}$ [116, 117]. Partitioning of this labeled contaminant can be measured after extraction of each compartment with the addition of a “scintillation liquid” that converts the resulting gamma-rays to UV-VIS radiation. The drawback of this technique is that it involves work with radioactive isotopes.

Equilibrium dialysis is frequently used to determine $K_{DOC}$ values. In this method an aqueous solution of DOC and the pollutant is placed in a dialysis bag (sample chamber) with a certain cut-off weight. Because of this the pollutants bound to the DOC cannot go through the membrane. The dialysis bag is than placed in DOC-free water in the assay chamber. At equilibrium the concentration of the freely dissolved pollutants will be the same on both sides of the membrane. With the analysis of the pollutants the $K_{DOC}$ values can be determined [11, 108, 117-119]. Limitations of this method are that sorption of contaminants to the membrane and surfaces occurs (these can be eliminated
by control samples), and that it does not account for sorption that may take place involving DOC molecules below the cut-off size of the membrane.

Several other methods have been used to determine sorption constants that are not mentioned here but are summarized in the review by Krop [103]. However, there are some especially interesting techniques like the “inverse GC”-method where humic and fulvic acids are applied to a sorbent, and the retention time is correlated with the distribution constant [104]. The hollow-fiber technique is also very interesting in that it is somewhat like a “liquid-filled-SPME” and is particularly useful for more hydrophilic contaminants [120].

In Papers II and III, we focused on solid phase microextraction (SPME) as the methodology for the determination of $K_{\text{DOC}}$ values. This technique will therefore be discussed more in detail below.

### 6.2. SPME and its advantages and limitations in determining DOC sorption

Solid phase microextraction is basically performed by immersing a polymer-coated silica fiber into a vial containing the analytes of specific interest [121, 122]. The fiber can either be directly immersed into liquid, or into the headspace, or used in a flow-through design (in-tube SPME) [123]. In Papers II and III SPME was performed in the direct immersion extraction mode. Some significant advantages of SPME as opposed to other sampling techniques are that it minimizes matrix effects and that it can be very efficiently coupled to chromatographic techniques either using thermal desorption to GC or liquid desorption to LC [124].

The idea behind using SPME for the examination of sorption of organic contaminants to DOC is based on two major attributes of this technique. First, the whole concept of SPME is based on its negligible-depletion sampling mechanism. This means that the amount of target contaminants that are
sampled from the bulk solution is so small that it does not affect the extant equilibrium distribution of an analyte in multi-phase systems [123]. Different perceptions of “negligible-depletion” have been proposed by authors where acceptable extracted percentages of the total amounts ranged from 1 % – 10 % [125-127]. In Paper II we have proposed a threshold of 5 %, and estimated depletion values in the range of 0.01 % – 5 % for all contaminants. Secondly, the immersed SPME fiber only takes up the unbound fraction of the analyte, which makes it representative of the freely dissolved concentration of the analyte (Figure 7).

![Diagram of SPME uptake](image)

*Figure 7: Uptake of phenanthrene by SPME in the presence of DOC. The freely dissolved fraction of phenanthrene gets taken up by the fiber, while the DOC sorbed fraction is unaffected.*

### 6.3. Theory of equilibrium and pre-equilibrium SPME

SPME can be used in equilibrium and pre-equilibrium sampling. One way is to wait until the equilibrium between the fiber and the sampled phase sets in. This is called equilibrium sampling. Equilibrium onset can vary from 30 minutes to several days depending on the target analytes [125, 128, 129]. In
equilibrium, the relationship between the amounts absorbed by the fiber and the free aqueous concentration can be given as follows [130]:

\[
\frac{V_{aq}K_{FW}}{K_{FW}V_{fiber} + V_{aq}}C_{aq,0} = C_{fiber}
\]

Where \(V_{aq}\) and \(V_{fiber}\) are the volumes of the sample and the fiber, \(C_{aq,0}\) and \(C_{fiber}\) are the starting analyte concentration in the sample and the equilibrium analyte concentration in the fiber, respectively. \(K_{FW}\) is the fiber-water distribution constant. If the volume of the sampled phase is much larger than the fiber itself, than \(K_{SW}V_{fiber} \ll V_{aq}\) thus:

\[
K_{FW}C_{aq,0} = C_{fiber}
\]

\[
n_{fiber} = C_{fiber}V_{fiber} = K_{FW}C_{aq,0}V_{fiber}
\]

Where \(n_{fiber}\) and \(V_{fiber}\) are the amount of the analyte in the fiber and the fiber volume, respectively. So in equilibrium conditions, if the sampled volume is large enough, the amounts taken up by the fiber are not dependent on the sampled volume, only the fiber volume and the fiber-water distribution constant. The distribution constant is defined by the activities of the analyte in the fiber and the sample. In case of organic contaminants in a sorption experiment, their concentrations lie in the ppb range, which is dilute enough to use concentrations instead of activities:

\[
K_{FW} = \frac{a_{fiber}}{a_{aq}} = \frac{C_{fiber}}{C_{aq}}
\]

In his book, Pawliszyn correlates \(K_{SW}\) values to chromatographic parameters on appropriately coated columns [130]. For fused silica – polydimethylsiloxane (PDMS) SPME fibers we have found that the contaminants of interest show a relatively good correlation with the octanol-water distribution coefficients (log \(K_{OW}\)) if measured after the onset of equilibrium (Figure 8). The slope was similar to what other authors have reported for PAHs, PCBs and PBDEs [131, 132].
The equilibrium $K_{SW}$ constants are affected by pH, temperature, salinity and matrix effects [130].

![Graph showing correlation between $\log K_{SW}$ and $\log K_{OW}$](image)

**Figure 8:** Correlation of $\log K_{OW}$ and $\log K_{SW}$ values for PDMS SPME fiber measured at equilibrium conditions during method development for 21 non-ionic organic contaminants.

The kinetics of SPME extraction of hydrophobic organic contaminants ($\log K_{OW} > 3.5$) are mostly determined by the water boundary layer (WBL), which is a thin aqueous layer that “sticks” to the surface of the fiber. In this layer the transport mechanisms are limited to molecular diffusion [129, 130]. The uptake speeds of these analytes are therefore primarily determined by the thickness of this WBL. In order to achieve faster uptake, the water boundary layer thickness can be reduced by agitating the system by stirring or ultrasonication. The overall thickness of the WBL (and thus the extraction speed) is determined by the diffusion constant (D) of the analyte in water and the agitation speed [130]. A usual extraction profile is presented in Figure 9.
As mentioned above, for some compounds equilibration times can be measured in hours or days. To achieve shorter extraction times SPME can be performed in pre-equilibrium mode. These two techniques have been compared in several studies [125, 128, 129]. The relationship between the sorbed amounts and the initial concentration of the analytes in the sample was established as [125]:

\[
C_{f,t} = \frac{k_1}{k_2} C_{w,0} (1 - e^{-k_2 t})
\]

The derivation of this relationship and the theoretical aspects of pre-equilibrium SPME are discussed in the introduction to Paper II. In order to state that this method is valid for the determination of DOC-water distribution constants, the uptake kinetics have to be the same for samples that contain DOC and the parallel samples without DOC. Violating this condition can lead to
experimental artifacts due to altered transport mechanisms ($D_s$ constants) in the WBL facilitated by DOC molecules at higher concentrations [129, 133].

Calibration of the SPME extraction for free contaminant fraction determination can be performed in different modes such as external/internal standard methods and standard addition [134]. However, for sorption experiments the external standard method is almost exclusively applied. The internal standard method is not useful because the internal standard can also interact with the DOC and so yield false response factors. The standard addition method will calibrate for the total amount of contaminants, not the freely dissolved amounts [129].

Negligible-depletion SPME has been one of the most popular techniques that researchers have been using to determine DOC and pore water DOC and particulate OC distribution constants for a wide variety of compounds starting with PAHs, PCBs, PBDEs [99, 103, 135-139] and extending to other compound classes like pyrethroids, pesticides, pharmaceuticals, bisphenol-A, musk and other organic compounds [140-144].

6.4. Effect of solution chemistry on sorption ($K_{DOC}$)

Change in pH affects sorption from the contaminant side only if the compounds are ionizable [120, 145, 146]. For neutral compounds, the change in pH mostly brings about changes in the structure of DOC such as protonation of the phenolic and carboxylic groups that reduces the hydrophilic nature of the DOC constituents, thereby forcing them to take up a more confined conformation [13]. Sorption of organic contaminants therefore decreases because of available sites becoming hindered by the structural alteration. This effect is not always measurably significant [103].

The effect of salinity or ionic strength is much more relevant to sorption of contaminants to DOC in a brackish water environment like the Baltic Sea
where the mean salinity of the pelagic water ranges from 3 ‰ to 8 ‰ from north to south. Previous studies have shown that sorption of pesticides to humic acid decreases with increased added amounts of salt [147]. Sorption of chlorpyrifos and DDT decreased most between salt concentrations of 1 ‰ – 5 ‰ in the presence of humic acid within the range of 3 mg/L – 25 mg/L carbon. Decreased $K_{DOC}$ values of phenanthrene have also been shown with increased salinity [13], the amount of decrease in $K_{DOC}$ being directly proportional to the charge state of the cations in the solution.

The presence of cations has been shown to have a large effect on the partitioning behavior of organic contaminants. The salting-out effect of salts has been reported but, on the whole, the largest differences are caused by the presence of divalent cations in the water [11, 12, 14]. This has mostly been explained by the ability of multivalent (Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Al$^{3+}$, Fe$^{3+}$, etc.) cations to "coil up" the macromolecular constituents of DOC through complexation, so making their hydrophobic sites less accessible for sorption [148]. The charge state of cations also takes effect as the overall ionic strength depends on them in ideal solutions.

$$I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$$

The aggregation of DOC macromolecules is even more pronounced in the presence of cations that are able to form multiple bonds which can then "coil up" these molecules more efficiently into even more confined structures [11-13]. The salinity effect is not expected to make a significant difference at the salinity range (3 ‰ – 8 ‰) found in the pelagic Baltic Sea.

6.5. Modeling of sorption

Many efforts have been made to predict the sorption of organic contaminants to DOC. Quantitative structure-activity relationships (QSARs) are
generally used for the prediction of different properties (boiling point, biological activity or partition coefficient) of certain molecules based on their chemical structure. Generally, the structural information is translated to molecular descriptors and it is mostly described as having a linear relationship with a selected property of the chemical. Perhaps the most widely used QSAR to model \( K_{DOC} \) is based on a linear solvation free-energy relationship (LSER) with the octanol-water partitioning coefficient [100, 109]. This \( K_{OW} \) value represents the hydrophobicity of a compound. The correlation between \( K_{DOC} \) and \( K_{OW} \) is given by the slope (a) of the linear regression of the \( K_{DOC} \) against \( K_{OW} \) plot.

\[
K_{DOC} = aK_{OW} + b
\]

Several different values have been reported for the \( a \) and \( b \) values, but good correlations can only be reached within a certain group of compounds with similar properties [149]. The historical Burkhard equation is probably the most general model that predicts \( K_{DOC} \) from \( K_{OW} \) values with 95 % confidence [109]. Several other molecular descriptors have been used in literature. For example the hydrogen-carbon and hydrogen-oxygen ratio retrieved from elemental analysis or \(^{13}\)C NMR data, which gives structural information about the aromaticity or polarity of a compound.

However, such single-parameter LSER only assumes that there is a linear relationship between two sets of data and do generally not provide mechanistic understanding of the partitioning process. In practice, the predictive power of single-parameter LSERs is limited to groups of compounds with a structural similarity. There is no single parameter that can describe all the molecular interactions that are important for the partitioning processes. Thus, for different groups of compounds different regressions must be made.

For a better understanding and more precise prediction of partitioning processes, polyparameter LSERs (PP-LSERs) can be used. In these relationships, free energy exchange is translated into energy components of
different types of intermolecular interactions (dispersion, inductive, Keesom- and Debye-forces, and hydrogen bonding). Using these parameters as corresponding to different interactions (interaction parameters), and different regression coefficients as adjustable parameters for the energy values, a multi-parameter linear model can be constructed [150]. In practice the interaction parameters are replaced by different quantitative descriptors based on experimental data (for example, known partitioning coefficients between two phases or retention factors from LC) or quantum mechanical calculations.

The parameters in Abraham’s general solubility model were originally used to gain a mechanistic understanding of retention in reversed phase LC [151, 152], whereas Hansen’s solubility parameters are based on the theory that “like dissolves like” and describes the molecular and bonding similarities of different molecules. This theory is mostly used to describe additive solubility in polymers.

Abraham solvation parameters are generally used to determine the distribution of organic pollutants between a pair of environmental compartments. Investigations have examined the distribution of polychlorinated biphenyls [153] and various other compounds [151, 154]. It has also been used to predict various physical-chemical properties of polychlorinated biphenyls (PCBs) [155].

The derivation of Abraham’s solubility parameters comes from a linear combination of different molecular descriptors, which are all derived from free-energy related properties. The approach of this method is to model those partitioning processes that are difficult to measure, based on easily measurable properties.

For the prediction of $K_{DOC}$ for organic compounds of different sizes and polarities the following equation can be used:

$$\log K_{DOC} = c + eE + aA + bB + sS + vV$$
The perception of the solvation process is that it consists of three major steps (Figure 10). Step 1 is cavity formation within the solvent (water and DOC) that involves the “deactivation” of the intermolecular interactions. Step 2 is the insertion of the solute molecule (contaminant) into this cavity. Finally, Step 3 is the “activation” of the attractive intermolecular forces between the solute and the solvent [156].

Figure 10: The three-step process of solvation of phenanthrene in a liquid.

The Abraham parameters for the solutes are represented with capital letters E, A, B, S and V. The constant c and solvent descriptors (e, s, a, b, and v) are determined by multi-parameter linear regressions using the experimentally derived log $K_{DOC}$ values for a series of compounds with predetermined or predicted Abraham parameters. Their physical meanings are summarized in Table 2 [156, 157]. The value of the coefficients describes the extent to which a certain molecular descriptor governs the DOC-water distribution process.
**Table 2: The physical qualitative interpretation of the molecular descriptors named in the Abraham LSER equation.**

<table>
<thead>
<tr>
<th>Contaminant-specific parameters</th>
<th>DOC-specific parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>Excess molar refraction (dispersive interactions)</td>
</tr>
<tr>
<td></td>
<td>$e$ Difference in excess molar refraction between water and DOC (interactions through</td>
</tr>
<tr>
<td></td>
<td>induced dipole that is not included in parameter $s$)</td>
</tr>
<tr>
<td>$A$</td>
<td>Hydrogen bond acidity (hydrogen bond donating ability)</td>
</tr>
<tr>
<td></td>
<td>$a$ Difference in hydrogen bond basicity between water and DOC (hydrogen bond</td>
</tr>
<tr>
<td></td>
<td>accepting ability).</td>
</tr>
<tr>
<td>$B$</td>
<td>Hydrogen bond basicity (hydrogen bond accepting ability)</td>
</tr>
<tr>
<td></td>
<td>$b$ Difference in hydrogen bond acidity between water and DOC (hydrogen bond donating</td>
</tr>
<tr>
<td></td>
<td>ability).</td>
</tr>
<tr>
<td>$S$</td>
<td>Polarity (Keesom and Debye interactions)</td>
</tr>
<tr>
<td></td>
<td>$s$ Difference in interactions through permanent and induced dipole interactions</td>
</tr>
<tr>
<td>$V$</td>
<td>Molar volume (proportional to molecular volume/surface)</td>
</tr>
<tr>
<td></td>
<td>$v$ Differences in required energy to create a cavity in water and DOC phase.</td>
</tr>
</tbody>
</table>

* The letters $F$ and $U$ stand for favorable and unfavorable nature of the mentioned interactions.

Abraham LSERs have previously been used to provide molecular descriptors for commercial humic and fulvic acids in surface water and pore water [158, 159]. They have not previously been used to describe spatial differences in the quality (sorption properties) of naturally occurring DOC.
7. Determination of the bioavailable contaminant fraction using passive sampling

A number of exhaustive methods have been used to determine whole water concentrations of contaminants [160]. It is generally understood that uptake of contaminants by microorganisms takes place in unbound, freely dissolved form. For organisms on higher trophic levels, biomagnification has to be included to evaluate bioaccumulation factors. The measurement of this free fraction is therefore the most informative and useful quantity for bioavailability studies. Passive samplers are often used to determine the free fraction of organic contaminants in water environments [161-165]. These samplers are operated solely by the diffusion of compounds through a membrane without any active control of sampling rates. A semi-permeable membrane device (SPMD) is the type of passive sampler that is used to determine the time-weighted average concentration of free hydrophobic compounds. This sampler mimics the bioconcentration process that takes place in fatty tissues of organisms. A high molecular weight lipid (most commonly triolein) is placed inside a semi-permeable polyethylene membrane (> 600 Da). This enables the diffusion of small pollutant molecules into the inside of the sampler and excludes macromolecules, particles and microbes.

Sampling time may last from days to several months depending on experimental design. After sampling, the pollutants are extracted (dialyzed) from the lipid phase to an organic solvent (e.g. hexane) and after cleanup and other purification methods the concentrations can be determined. This sampler can be used to determine the free concentrations of hydrophobic pollutants such as PAHs, PCBs, organochlorine pesticides, some organophosphates and various other apolar compounds with good reproducibility. For more hydrophilic compounds another passive sampling technique, polar organic chemical integrative sampler (POCIS), can be used. The principle of this method is analogous to SPMD. A sorbent is kept between two microporous
polyethersulfone membranes that can facilitate the transport of ionic and other polar organic compounds. The sorbent can be varied depending on what the model compounds are. The main difference between the sampling mechanisms is that SPMDs are based on partitioning and give average concentrations, whereas POCIS is based on sorption and yields integrative results over the exposure time.

In an analogous way to SPME, SPMDs can be operated in equilibrium and kinetic modes. Because the sampled medium is not being actively stirred and agitated, and because the volume of the sampler is large, the onset of equilibrium can be measured from weeks up to years for very hydrophobic compounds [166]. For monitoring contaminants in water, both sampling modes are usually used. The ultimate goal in passive sampling is to calculate the free aqueous concentration of a contaminant based on the amounts measured in the sampling device. Based on the general solution of the differential equation given for the concentration of a compound in the sampler at a certain time point [166], the following equation can be written to establish the relationship between the water concentration ($C_{\text{water}}$) and the amounts in the sampler ($N_S$):

$$C_{\text{water}} = \frac{N_S}{K_{SW} V_S [1 - \exp \left( \frac{R_S t}{K_{SW} V_S} \right)]}$$

The parameters $K_{SW}$ and $V_S$ are the volume averaged sampler-water partitioning constant (for the triolein and membrane) and the sampler volume, respectively. $R_s$ is the sampling rate, which is proportional to the mass transfer coefficient through the system, and $t$ is the sampler exposure time. The term $R_{st}$ is proportional to the sampled amounts and inversely proportional to the concentration gradient in the sampler. As equilibrium is approached, the concentration gradient decreases and the term in the exponent tends towards zero, giving therefore:
In kinetic sampling the concentration gradient is large and the sampled amounts are small, so the water concentrations can be determined by:

\[ C_{\text{water}} \approx \frac{N_S}{K_{SW} V_S} \]

\[ C_{\text{water}} \approx \frac{N_S}{K_{SW} V_S} \left( \frac{R_S t}{K_{SW} V_S} \right) \]

\[ C_{\text{water}} \approx \frac{N_S}{R_S t} \]

In equilibrium sampling, the water concentrations depend on the equilibrium partitioning constant. For kinetic sampling, the sampling rates must be determined. It is therefore important to have proper calibration methods to determine sampling rates. Calibration on SPMDs can be done by different methods that include static exposure, where the samplers are being exposed to a water mass with a certain amount of spiked contaminants [167, 168]. Two other methods are static renewal [169] and continuous flow calibration [170, 171], which is done in a similar manner but with an infusion of water with a constant concentration of contaminants. In situ calibration has been developed in order to enable simultaneous calibration and sampling for SPMDs. This involves the application of so-called performance reference compounds (PRCs) as standards that approximate the mass transfer of organic contaminants between the SPMD and the water. Only compounds that do not occur naturally can be used as PRCs. Such compounds include deuterated PAHs and certain PCB congeners [168, 172]. The sampling rates can be calculated from the elimination rate constants \((k_e)\) using the following equations:

\[
k_e = -\frac{\ln C / C_0}{t}\]
Where \( C \) and \( C_0 \) are the measured and initial concentrations of the PRCs in the sampler, respectively. The sampling rates, and thus the water concentrations, can be calculated using the elimination rate constants \( k_e \), equilibrium sampler-water distribution constants \( K_{SW} \) and the sampler volume \( V_S \) [166]:

\[
R_S = k_e K_{SW} V_S
\]

Since the \( K_{SW} \) values are not the same for all measured contaminants – in fact they correlate quite well with \( K_{OW} \) for SPMDs using polydimethylsiloxane (PDMS) membranes – in practice several PRCs are used that cover the whole range of sampler-water equilibrium distribution constants.
8. Instrumental analysis

8.1. On-line SPME sample preparation system

One of the major tasks of this PhD thesis was to develop and apply a method for the in situ determination of DOC-water distribution constants of various Baltic Sea water samples. SPME was selected due to its advantages described above; most importantly it enabled reliable analysis of free and sorbed pollutant concentrations without disturbing the equilibrium in the system. It is also easy to automate the method.

Two different types of fibers were selected. For the more hydrophobic contaminants, a 7 μm thick fiber was used with a fused silica core and polydimethylsiloxane (PDMS) coating. For the aromatic and more polar compounds we used a polystyrene-divinylbenzene (PS-DVB) fiber with 65 μm thick PDMS coating. The largest SPME vials were selected in order to minimize the depletion of the solutions, and the contaminant concentrations were all below their respective water solubility.

Figure 11: The Gerstel MPS-XL on-line SPME sample preparation system and its main building blocks.
The performance of manual SPME extraction was initially evaluated and was found to be unsatisfactory. A Gerstel MPS XL (Gerstel GmbH & Co.KG) and a cooled inlet system (CIS 4) were therefore used for on-line SPME. The layout of the system is presented in Figure 11.

Efficient mixing of the solutions was carried out using the agitator unit in order to decrease the water boundary layer thickness and thus increase the uptake speed of the contaminants. The software Maestro from Gerstel included a so-called PrepAhead function that enabled the incubation and extraction of up to six samples simultaneously. This feature decreased the total analysis time and is discussed further in Paper II. The performance details of the automated on-line SPME-GC-MS method is discussed in Paper II, but it is worth mentioning here that the raw data repeatability was < 11 % RSD (n = 4) for all examined contaminants.

8.2. Gas chromatography – mass spectrometry

For the vast majority of GC-MS analyses performed in this work a Leco GC-HRT system was used. This system has a unique “folded” flight tube design (Figure 12.) that enables a mass resolution above 50,000 full width at half measure for the ion m/z 219.

In time-of-flight mass spectrometers the m/z ratio is proportional to the square root of the time it takes for the ion to reach the detector from the extracting electrodes in the mass analyzer. The more time the ions spend in the flight path, the more they can separate. The GC-HRT can be operated at two different modes called high resolution (HR; < 25,000 FWHM) and ultra-high resolution (UHR; < 50,000 FWHM), with a flight path of 20 m and 40 m, respectively. In the UHR mode the mass range may have to be limited to avoid the slow, heavier ions from an extraction pulse to be overtaken by the fast, light ions of the subsequent pulse. The procedures and thresholds for the GC-HRT optimization are described in Papers I and II.
Figure 12: The "folded" flight path design of the LECO-HRT-TOF-MS. The analytes enter the flight tube from the ion source. Gridless mirrors and the periodic ion lenses build up the folded flight path structure which adds symmetry to the ion path that compensates for ion dispersion and results in better mass accuracy. The detector collects the ions and is followed by the data acquisition system. The figure was taken from a presentation by Peter Haglund as it had been found on the Leco website [173].

The software used for the operation of the Leco GC-HRT is called ChromaTOF. In most cases in these studies, target analysis has been performed. The respective feature for this workflow in the software is called “target analyte finding”. This function uses the elemental compositions of certain fragments of the target compounds and calculates the exact masses. Quantification of ions was done by generating extracted ion chromatograms from the raw data by opening up a user-defined mass window (e.g. 0.05 Da) around these theoretical exact masses. The elemental composition of fragment ions was obtained by comparing experimental data with tentative annotations from the NIST MS spectra interpreter, or by deriving the structure by manual spectra interpretation. Other isotope peaks and lower intensity peaks in the corresponding target analyte spectra were used to confirm identities.
9. Summary of experimental studies

9.1. Paper I. Interference of isotope-labeled standards

One of the first projects planned involved the passive sampling of Baltic Sea water using SPMDs and the determination of free aqueous concentrations of hydrophobic organic pollutants. During the analyses of PAHs we discovered spectral interferences of isotope-labeled performance reference standards (PRCs).

When quantifying US EPA priority PAHs, expensive $^{13}$C-labeled internal standards are used for corrections due to instrument instability and losses in sample treatment. The PRCs used to obtain accurate sampling rates (and thus accurate aqueous concentrations) of contaminants in kinetic sampling mode of SPMDs are often, however, inexpensive perdeuterated PAHs (which are generally used in larger amounts).

In **Paper I**, we report a significant systematic error (positive bias) that originates from the spectral overlap of the molecular ion of the $^{13}$C-labeled internal standard by PRC fragments formed by de-deuteration of perdeuterated PAHs. In the ion-source, PAHs go through subsequent gas-phase losses ($N$ times) of hydrogen that cause the appearance of peaks $n^*(-1)$ m/z distance from the molecular ion. These peaks correspond to $[M - nH^{+*}]$. In case of $^{13}$C-labeled ISs, where the difference in the m/z of molecular ions is 6, this phenomenon does not lead to spectral overlap. But in the case of perdeuterated compounds $[M - nD^{+*}]$ a loss of deuterium appears as $n^*(-2)$ m/z on the mass spectrum. In the case of acenaphthene, fluorene, phenanthrene, chrysene and benzo(e)pyrene, whose perdeuterated forms are used as PRCs, a loss of 2 to 4 deuterium atoms leads to spectral overlap of their respective $^{13}$C-labeled molecular ions. This led to a 1.7 % – 13.6 % systematic error when quantifying PAHs in a real sample. We used ultra-high resolution MS to quantify and subsequently eliminate these errors.
9.2. Paper II: SPME method development

A reliable pre-equilibrium SPME-GC-MS method was developed for the determination of DOC-water distribution constants. All the requirements for the kinetic sampling to be valid were carefully examined. Uptake rates were recorded and were found to be unchanged in the presence of 10 mg carbon/L DOC. The fiber surface was examined using fluorescence microscopy, which showed no discoloration and no pyrolysis products were found.

Of the 37 model contaminants used for the experiments, this method provided valid log $K_{DOC}$ measurements for 30 of them at different Nordic Reference Fulvic Acid (NRFA) concentrations. Two criteria were proposed in Paper II to establish reliable DOC working ranges: 1) that the average of the free concentrations measured in samples with and without NRFA should be statistically significant from each other ($p < 0.05, 95\% \ CI$); and 2) the amount of contaminant sorbed to NRFA should be $>10\%$ of the total amount added to the system.

Most of the contaminants proved to have valid log $K_{DOC}$ values within the 10 – 100 mg carbon/L concentration range. Even though the contaminants with lower log $K_{DOC}$ values sometimes showed less than 10 % DOC-sorbed amounts at lower NRFA concentrations, in particular 5 mg carbon/L, their log $K_{DOC}$ values were often close to those measured at higher NRFA concentrations. Thus, the good repeatability of the raw data enabled the log $K_{DOC}$ measurements of contaminants with weaker sorption to DOC at environmentally relevant DOC concentration levels to be used.

The limitation of this method was apparent at NRFA concentrations above 100 mg carbon/L. The log $K_{DOC}$ values were clearly underestimated at 200 mg carbon/L. We found support in the literature for the notion that change in uptake kinetics and in DOC structure may lead to experimental artifacts [103, 174, 175].
9.3. Paper III: Dissolved organic carbon characterization and log $K_{DOC}$ determination throughout the Baltic Sea

A sampling cruise was conducted to examine the differences in DOC quality and sorption of the model organic contaminants. The sampling points and their respective salinity and DOC concentration are given in Figure 13 and the sampling is described in detail in Paper III. The timing of the sampling was arranged such that sampling took place after the cyanobacterial bloom season – which usually ends in mid-August – in order to avoid any large effect due to autochthonous DOC.

![Figure 13: Spatial differences in salinity and DOC concentrations throughout the Baltic Sea. Salinity (‰) (blue figures left of the oblique) and the log $K_{DOC}$ values of different organic contaminants from north to south (red figures right of the oblique).](image)

The Baltic DOC was characterized with $^1$H-NMR (after isolation) and SUVA (in situ). The results (Figure 14) revealed increasing aromaticity of the
DOC towards the northern parts of the Baltic Sea, indicating an increasing ADOC contribution to the bulk DOC.

![Graphs showing measurements of DOC aromaticity throughout the Baltic Sea using UV absorption spectroscopy at 254 nm (SUVA\textsubscript{254}) and \textsuperscript{1}H-NMR spectroscopy (ratio of peak areas of aromatic and aliphatic protons, normalized to the DOC content). The bottom graph presents the correlation between the two parameters.](image)

**Figure 14:** Measurements of DOC aromaticity throughout the Baltic Sea using UV absorption spectroscopy at 254 nm (SUVA\textsubscript{254}) and \textsuperscript{1}H-NMR spectroscopy (ratio of peak areas of aromatic and aliphatic protons, normalized to the DOC content). The bottom graph presents the correlation between the two parameters.

The method described in Paper II was then used to determine log $K_{DOC}$ values *in situ* for the model chemicals in the Baltic Sea along a north-south gradient. Figure 15 shows a decreasing trend in log $K_{DOC}$ for the majority of contaminants. Planar halogenated compounds such as pentachlorobenzene or 2,4-dibromoaniline exhibited gradually decreasing log $K_{DOC}$ values going from north to south. On the other hand, compounds with higher hydrogen-bond donor/acceptor capabilities such as octylphenol and alkyl organophosphates showed no remarkable differences (Figure 15). The log $K_{DOC}$ of PAHs showed
no significant differences in Bothnian Bay to the Bothnian Sea and dramatically decreased towards the Baltic Proper.

![Figure 6: Spatial differences in the log \(K_{DOC}\) values of different organic contaminants from north to south. PeCBz stands for pentachlorobenzene.](image)

The log \(K_{DOC}\) values were then used to determine the DOC-specific molecular descriptors using LFERs for DOC samples in the Baltic Proper and the Bothnian Bay. The main differences between the sorption characteristics of the two DOC pools where that the Baltic Proper DOC yielded a higher contribution from the hydrogen bond terms \((a, b)\); whereas DOC in the Bothnian Bay had a higher contribution of the dipolarity term \((s)\), which is related to aromaticity through induced-dipole interactions. This can be explained by increased photo-oxidation of DOC in the south and its higher hydroxyl group content.

In summary, sorption of organic contaminants to DOC changes spatially in the Baltic Sea. Most importantly, these differences can be deduced
from the increased aromaticity in the north; but higher polarity and the more oxidized state of DOC in the south also plays a role in these changes.

9.4. Paper IV. Environmental fate of organic contaminants in brackish water mesocosms

Another major part of this PhD project was to examine the distribution of environmental contaminants in simulated ecosystems at the end-points of possible climate change scenarios predicted by regional models described in Chapter 4.1. For this purpose, an aquatic mesocosms experiment was planned. Mesocosms are normally used to investigate roles of certain environmental stressors on certain species or ecosystems in a controlled environment. Mesocosms in the field of environmental chemistry have previously been used primarily for toxicity studies of agricultural chemicals (pesticides), the effects of chemicals originating from oil spills, or, rarely, for environmental fate (mostly biodegradation) studies [176-182].

Figure 16: The sampling procedures (weekly and at the conclusion of the experiment) for one mesocosm are given on the left of the dashed line. On the right side, the full 2 x 2 factorial experimental design of the brackish mesocosm experiments is summarized. Each colored square stands for a separate treatment. The illustration represents the actual experimental setup.
The experiment took place in the Umeå Marine Sciences Centre (UMF) located outside Norrbyn. Brackish mesocosms were applied to model pelagic ecosystems at present (15 °C and 4 mg/L DOC) and future states (18 °C and 6 mg/L DOC) in a full factorial design with 3 parallel treatments (Figure 16).

The free and total sub-0.7 μm aqueous concentrations of the contaminants were analyzed weekly; at the conclusion of the experiment the different size fractions were separated and analyzed (Figure 16). The detailed description on the experimental design and the methods are discussed in Paper IV.

Figure 17: The dissolved amounts of selected organic contaminants in brackish water mesocosms.

The environmental fate studies of the contaminants in brackish mesocosms showed a great deal of interesting tendencies. Firstly, the weekly dissolved contaminant concentrations rapidly decreased from the day of addition. The weekly and final dissolved contaminant concentrations were
monitored but, surprisingly, did not show statistically significant differences by treatment (this was most probably due to large variations among the replicates – Figure 17).

A comprehensive contaminant analysis was performed on each size fraction at the conclusion of the experiment. A mass balance was generated using the total amounts of contaminants in each compartment of the model ecosystems. The most obvious tendencies can be observed in the amounts of particle-sorbed contaminants (Figure 18).

For most contaminants the total amounts increased with higher organic carbon content, but they also decreased at higher temperatures. The same tendencies were observed when using concentration data (Figure 19).

Losses due to bio- and photodegradation are very hard to estimate. We did, however, estimate the direction of air-water exchange, using complementary passive air sampling data, thereby approximating the losses due to volatilization.
Figure 18: Total contaminant amounts in the 90 μm particulate size fraction (above) and in the sediment (below).
Figure 19: The contaminant concentration in sediment, normalized to dry weight basis.

Figure 20 provides a qualitative summary of the main outcome of this experiment. Clearly, higher organic carbon loadings promote sorption and sedimentation due to the introduction of higher amounts of particulate matter into the systems - providing additional surfaces onto which hydrophobic contaminants can adsorb (left block of data). Increased temperatures result in higher losses of contaminants, which is probably due to increased hydrolysis and photolysis degradation rates, enzyme activities and net volatilization rates, which all are directly proportional to temperature (central block of data). Combined effects of higher temperature and organic carbon load lead to intermediate results, with higher losses or more particle association, depending on compound properties. Both processes lead to smaller amounts of contaminants in the dissolved phase (right block of data).
Figure 20: Qualitative summary of mesocosm results. From left to right: The effects of increased organic carbon content, increased temperature and the combined effect of both to the amount of contaminants found in different compartments. “Diss” stands for dissolved (the total amount of contaminants in the aqueous phase), “part” stands for particulate, which is the sum of the amount of particle-sorbed contaminants in all size fractions. “Sed” stands for sediment, which corresponds to the contaminant amounts measured in the sediment. The “lost” fraction was calculated by summing all measured amounts over all compartments, and subtracting that from the spiked amounts.
10. Concluding remarks

As has been shown in other studies, the “brownification” of the water has already started as a result of increased precipitation in the northern Baltic Sea [183, 184]. It has also been found during the course of this study that there are clear spatial differences in the aromaticity and the overall quality of the DOC. There are also differences in the sorption of aromatic contaminants throughout the Baltic with a tendency for gradients to decline when moving from north to south. These differences are most probably due to variation in DOC quality rather than to salting out effects, since the largest differences in log $K_{DOC}$ values were found between the Bothnian Sea and the Baltic Proper where the change in salinity is not spectacularly large ($5 \text{‰} – 7 \text{‰}$). This view is supported by our modeling results, which generally indicate increased hydrogen bond acidity and basicity of the DOC present in the Baltic Proper.

Overall, the higher sorption capacity of the DOC in the northern Baltic Sea leads us to suppose that the organic contaminants will tend to partition more to DOC in this environment. However, it is too early to draw conclusions about whether or not it will result in higher bioaccumulation of these contaminants in biota as this process largely depends also on the types of food-web that develop in these areas. For instance, in phytoplankton-based food-webs the uptake of contaminants begins with the freely dissolved fraction; in the presence of such an ecosystem, therefore, the inflow of DOC will decrease the uptake of contaminants. On the other hand, DOC serves as a food source for bacteria and if food-webs develop based on bacteria, the uptake of contaminants on the microbial level might result in increased bioaccumulation in higher trophic levels. Bacteria-based food webs often include an extra trophic level in the form of grazers, which could lead to higher biomagnification.

The effect of higher temperatures will tend to result in higher volatilization, and higher biodegradation rates of organic contaminants by
organisms, as these processes function in direct proportion to temperature. This has already been discussed at length in connection with climate change. Our model ecosystem experiments confirmed that, indeed, the amounts of contaminants decreased at higher water temperatures. We can also conclude that the effect of higher inputs of terrestrial organic carbon may result in increased sedimentation and, in general, increased retention of organic contaminants in the Baltic Sea. These processes seem to be competitive.

It is likely that contaminant distribution will be affected by future climate change, but the complexity of all these involved processes cannot be underestimated. Even though the action of two simple but relevant climate change factors seems to produce clear trends in the pattern of changes that they induce in the distribution of contaminants, when combined they produce varying trends with no clear patterns. It is therefore very difficult to conclude any kind of collective behavior for organic contaminants. The environmental fate of each individual compound will probably ultimately depend on its physicochemical properties and the climate change factor to which it is most sensitive.
11. Future needs and recommendations

The power of ultrahigh resolution mass spectrometry has been demonstrated in the thesis. It can eliminate spectral interferences in the determination of PAH concentrations using passive sampling devices. This technique could be utilized for more precise determination of free aqueous concentration of this group of chemicals and for improved assessment of their bioavailable fraction.

The limitation of SPME for determination of DOC-water partitioning constants of compounds with lower affinity to DOC was clearly apparent. In order to improve the reliability of the $K_{DOC}$ distribution constants the DOC concentration could be increased. However, in Paper II it was shown that at concentrations above 100 mg carbon/L the log $K_{DOC}$ values started to deviate for most contaminants. A good alternative could be to increase the sampling volume and to use integrative sampling methods, with long sampling times, to accumulate sufficient contaminant amount for a reliable instrumental determination.

It is necessary to extend the LFER models to a larger collection of organic contaminants to better understand the contribution of the various intermolecular interactions. Contaminants with functional groups missing in the current selection should be prioritized. In addition, improved data should be generated for the already studied contaminants with low log $K_{DOC}$ values; by using the above discussed sampling method.

Spatial differences in DOC quality among the Baltic Sea basins was unambiguously shown in Paper III. A comprehensive characterization of DOC samples taken along the studied north-south gradient could reveal structural differences between DOC from the three Baltic Sea basins. A “holistic” approach is recommended, including in-situ determination of bulk-DOC characteristics (e.g. fluorescence and SUVA), determination of other bulk-DOC
characteristics (e.g. elemental composition), and determination of molecular-level characteristics using advanced spectrometric and spectroscopic techniques (e.g. NMR and FT-ICR).

The ecosystem response to the climate induced changes in Baltic Sea temperature, mixing and water chemistry, including the concentration of DOC, needs to be better understood. Therefore, further controlled studies are needed for estimation of bioaccumulation in ecosystems with different structure. The studies should not include a large number of contaminants because of resource limitations and possible induction of adverse eco-toxicological effects.

Additionally, the reactivity of contaminants may also need to be investigated, as it may change with increasing DOC content. For example, contaminants that are susceptible to photodegradation may be “shielded” on a molecular level (electron transfer complexes) or indirectly by light attenuation. Alternatively, light may decompose DOC and produce high energy, unstable fragments that can react with and degrade contaminants, in so-called indirect photolysis reactions.
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