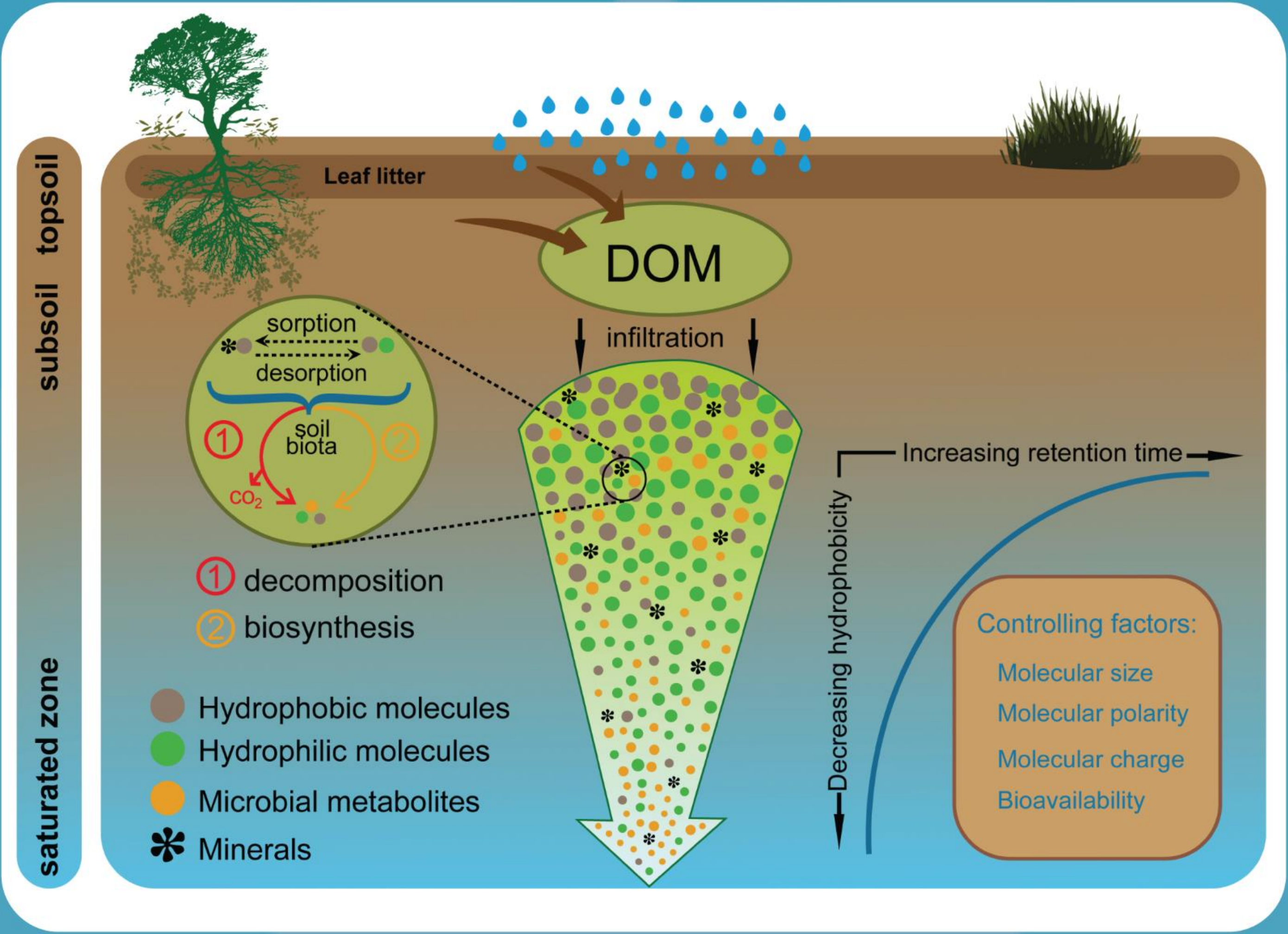

# Dissolved Organic Carbon in Groundwater Systems

## Francis H. Chapelle

THE GROUNDWATER PROJECT

# *Dissolved Organic Carbon in Groundwater Systems*

*The Groundwater Project*

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Thank you.

Francis H. Chapelle
Emeritus Scientist
United States Geological Survey, Retired
Little Mountain, South Carolina, USA

# Dissolved Organic Carbon in Groundwater Systems

The Groundwater Project
Guelph, Ontario, Canada
Version 4, January 2024

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THE GROUNDWATER PROJECT

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# Dedication

I dedicate this book to the late William Back of the U.S. Geological Survey, my teacher and mentor.

# Table of Contents

# The Groundwater Project Foreword

The Year 2022 marks an important year for groundwater because the United Nations Water Members and Partners have chosen the theme of this year's March 22 World Water Day to be: "Groundwater: making the invisible visible". The goal of the Groundwater Project (GW-Project) is in sync with this theme.

The GW-Project, a registered charity in Canada, is committed to contributing to advancement in groundwater education and brings a unique approach to the creation and dissemination of knowledge for understanding and problem-solving. The GW-Project operates the website https://gw-project.org/↗ as a global platform for the democratization of groundwater knowledge, founded on the principle that:

> *"Knowledge should be free and the best knowledge should be free knowledge." Anonymous*

The mission of the GW-Project is to promote groundwater learning. This is accomplished by providing accessible, engaging, high-quality, educational, copyrighted materials, free-of-charge online in many languages, to all who want to learn about groundwater. In short, providing essential knowledge tools for developing groundwater sustainably for humanity and ecosystems.

This is a new type of global educational endeavor in that it is based on the volunteerism of professionals from different disciplines and includes academics, consultants and retirees. The GW-Project involves many hundreds of volunteers associated with more than 200 organizations from 27 countries and six continents, with growing participation.

The GW-Project is an ongoing endeavor and will continue with hundreds of books being published online over the coming years, first in English and then in other languages, for downloading wherever the Internet is available. An important tenet of the GW-Project books is a strong emphasis on visualization via clear illustrations that stimulate spatial and critical thinking to facilitate the absorption of information.

The GW-Project publications also include supporting materials such as videos, lectures, laboratory demonstrations, and learning tools in addition to providing, or linking to, public domain software for various groundwater applications supporting the educational process.

The GW-Project is a living entity, so subsequent editions of the books will be published from time to time. Users are invited to propose revisions.

We thank you for being part of the GW-Project Community. We hope to hear from you about your experience with using the books and related material. We welcome ideas and volunteers!

The GW-Project Steering Committee

June 2022

# Foreword

Dissolved organic carbon (DOC) in groundwater is usually expressed as milligrams of organic carbon per liter of filtered water (mg/L). DOC in both ground- and surface water systems is made up of thousands of individual compounds that are difficult to identify. However, they rarely need to be identified for most groundwater applications and more inexpensive DOC measurements typically suffice. For that reason, DOC measurements are thus a routine component of many general-purpose groundwater analyses. DOC is present in all surface water and groundwater, but in groundwater the concentrations are small relative to their concentration in surface water. In uncontaminated (pristine) groundwater, DOC is generally much less than 1 percent by weight of total dissolved solids (TDS) present. However, DOC in groundwater is important despite the typically low concentration. Active bacteria of natural origin are present nearly everywhere in groundwater and they foster biochemical reactions that commonly influence or control groundwater quality (i.e., water usefulness). For example, the presence and nature of the DOC may be a controlling factor in the biochemical processes that govern the pH and oxidation-reduction state of the water. pH and oxidation-reduction state, in turn, governs concentrations of important water-quality constituents such as iron, manganese, chromium, arsenic and nitrate. It is useful to read the Groundwater Project book, *Groundwater Microbiology* (2021) as background for reading this book.

One reason for measuring the amount of DOC in groundwater is to determine whether the water has a normal concentration, with normal being between 0.3 and 1 mg/L. Exceptionally abnormal levels are above 5 mg/L. Abnormality usually indicates something important, for example the DOC may come from anthropogenic sources or from direct infiltration of water from a river or lake into an aquifer. Alternatively, it may indicate that agricultural or industrial activity has diminished DOC attenuation in water percolating through the vadose zone. Any of these situations would indicate the need to consider possible causes of contamination that would be worthy of further investigation.

The author of this book, Dr. Francis Chapelle is an emeritus Research Hydrologist at the United States Geological Survey where his research has focused on how microbial processes affect pristine and contaminated groundwater. He authored the textbook *Groundwater Microbiology and Geochemistry* that was published as a second edition in 2003 and he has received numerous awards for his life's work. The Groundwater Project feels fortunate that Dr. Chapelle freely provided this synthesis of humankind's knowledge of DOC for all who want to know more about groundwater.

John Cherry, The Groundwater Project Leader

Guelph, Ontario, Canada, August 2022

# Preface

Dissolved organic carbon (DOC) is a chemical constituent that is present in measurable quantities in virtually all groundwater. Because DOC typically consists of many thousands of different carbon-bearing compounds, its composition and its chemical and biological properties can be difficult to assess. This book summaries the most common sources of DOC to groundwater, the various analytical techniques for characterizing that DOC, the chemical and biological processes that control DOC concentrations, and the chemical and biological processes that affect the bioavailability of DOC. This book explains how and why DOC is important in the study of both pristine and contaminated groundwater.

DOC is an indicator of groundwater quality that can be determined by what have become standard methods. Other such indicator parameters are TDS (total dissolved solids), pH, Eh (redox potential) and EC (electrical conductance). These latter four indicator parameters are accepted as having broad usefulness in groundwater investigations and are commonly reported as part of the description of the nature of any groundwater. After 50 years of DOC research, there is sufficient literature to support DOC as an indicator parameter deserving a level of attention similar to these other indicator parameters.

# Acknowledgements

I deeply appreciate the thorough and useful reviews of and contributions to this book by the following individuals:

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I am grateful for Amanda Sills and the formatting staff of the Groundwater Project for their oversight and copyediting of this book. I am grateful to Doug Mackay (Professor Emeritus, Land Air and Water Resources, University of California Davis) for his creative content creations. I appreciate the final review provided by Ineke Kalwij (Kalwij Water Dynamics, Vancouver, British Columbia, Canada). I thank Eileen Poeter (Colorado School of Mines, Golden, Colorado, USA) for reviewing, editing, and producing this book.

Francis H. Chapelle

# 1 Introduction

As illustrated in Figure 1, dissolved organic carbon (DOC) is the fraction of organic carbon present in water defined as that which can pass through a filter with a pore size 0.45 micrometers and expressed as a concentration ($M/L^3$). The fraction remaining on the filter is called particulate organic carbon (POC) and is also expressed as a concentration ($M/L^3$). Dissolved organic matter (DOM) is a closely related term often used interchangeably with DOC. While DOC refers specifically to the mass of carbon in the dissolved organic material, DOM refers to the total mass of the dissolved organic matter. DOM includes the mass of elements other than carbon that are present in organic material, such as nitrogen, oxygen and hydrogen. DOC is a component of DOM and there is typically about twice as much DOM as DOC (i.e., the concentration of DOM is typically twice the concentration of DOC).

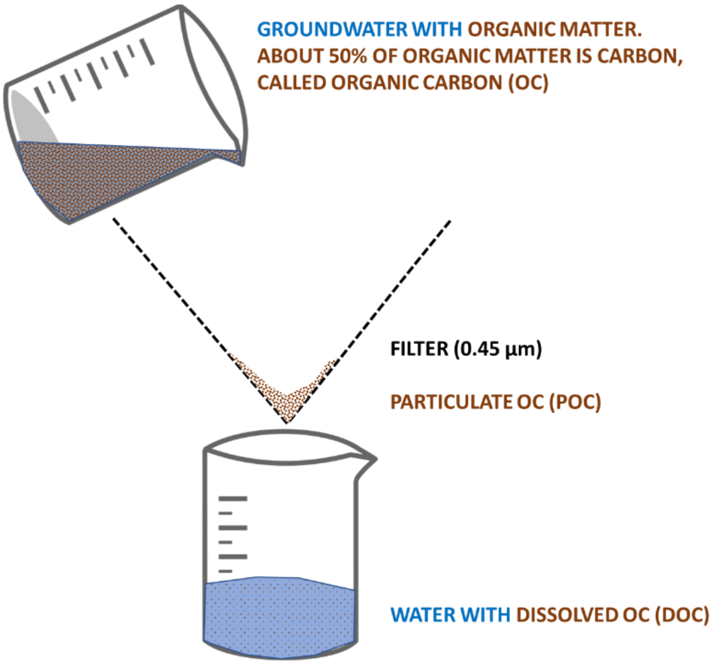


**Figure 1 -** Organic matter in water is typically about 50 percent carbon and is generally referred to as composed of two fractions, that captured by a 0.45 filter (POC) and that passing the filter – DOC – (Mackay, 2022).

Concentrations of DOC are commonly analyzed in investigations of water quality for both surface water and groundwater systems. For surface-water systems, this reflects the fact that DOC-removal treatment is frequently required to make the water suitable for human consumption (Kornegay et al., 2000). That is not, however, typically the case for groundwater. One of the defining characteristics of groundwater used for human water supply is that it generally lacks the higher DOC concentrations requiring removal treatment. The question can reasonably be asked, therefore, what is the practical usefulness of DOC measurements in groundwater? The answer is threefold.

Organic carbon, in its dissolved (DOC), particulate (POC), and adsorbed (AOC) forms as illustrated in Figure 2, determines the kinds and direction of reduction/oxidation (redox) reactions that can occur in an aquifer system. Those redox processes, in turn, determine concentrations of redox-sensitive chemical species such as dissolved oxygen, nitrate, ferrous iron, manganous iron, sulfide, and methane, all of which affect the real and perceived chemical quality (defined as its usefulness to humans and/or ecosystems) of groundwater. DOC-driven redox processes also affect the fate and transport of human-derived chemical contaminants such as petroleum hydrocarbons and chlorinated solvents. The dissolved, particulate, and adsorbed compartments of organic carbon interact dynamically with each other in groundwater systems, thus the bioavailability of DOC may indicate the bioavailability of particulate and adsorbed organic carbon.

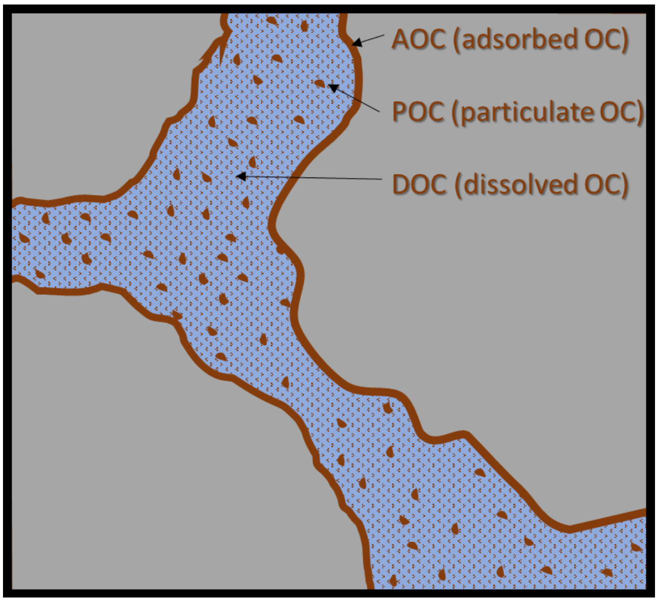


**Figure 2 -** Occurrence of AOC, POC and DOC in groundwater in a pore between grains or fracture walls within an aquifer. This is not a scale drawing, but meant to illustrate that typically there is a lot more organic carbon in AOC and POC than in DOC. AOC may not coat all particle surfaces nor coat them uniformly, as is illustrated here. Though not indicated, AOC may sometimes remobilize into the porewater, which increases DOC (Mackay, 2022).

*Particulate organic carbon* (POC) and *adsorbed organic carbon* (AOC) are always much more abundant than DOC in any given volume of aquifer material. In a study of ten shallow aquifers located throughout the continental United States (Chapelle et al., 2012b), measurements of total organic carbon (DOC + POC + AOC) were found to range from 50 to 10,000 milligrams of organic carbon per kilogram of aquifer material (mg/kg). To put those numbers in perspective, a typical clean white beach sand contains only about 50 mg/kg of total organic carbon. Given the density of quartz sand grains (1,631 kg/m$^3$), and assuming

50 mg/kg of POC + AOC, one cubic m (1 $m^3$) of sandy aquifer material contains 81,550 mg of organic carbon. Assuming a porosity of 0.3, that same volume of saturated aquifer material will contain 300 liters of water. If that water contains 5 mg/L of DOC (a relatively high value for groundwater), there is just 1,500 mg of organic carbon present as DOC. In other words, as illustrated in Figure 3, DOC in groundwater typically represents less than 1 percent of the total organic carbon present in any given volume of aquifer material.

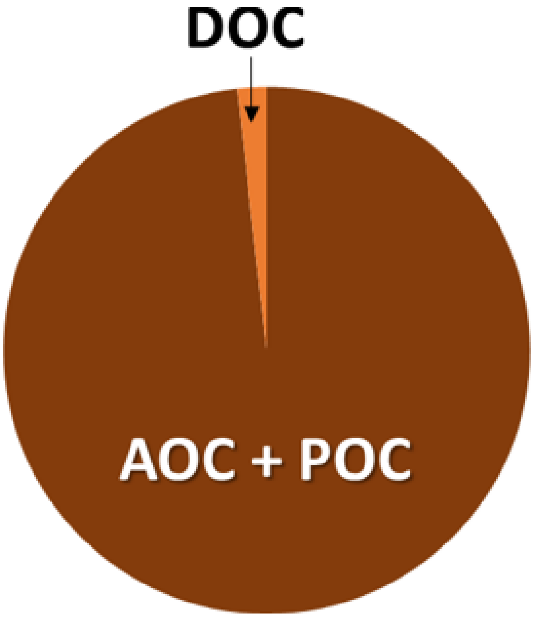


**Figure 3 -** DOC typically makes up a very small percentage of total organic carbon in a given volume of aquifer material. This illustration is for a clean white beach sand described in the text (Mackay, 2022).

If DOC represents such a small portion of the total organic carbon, how can DOC be of relevance to evaluating groundwater? Studies of DOC in groundwater published over the last fifty years reveal facts relevant to that question:

- DOC concentration in groundwater systems that are not under the immediate influence of surface-water sources are ubiquitously low due to a combination of DOC adsorption onto aquifer solids and biodegradation processes, while DOC of surface waters is typically 100 to 1000 times higher; and,
- DOC adsorption onto aquifer solids is reversible (Figure 4), so AOC can be remobilized as DOC suggesting that the chemical and biological properties of DOC can reflect those of POC and AOC.

Thus measurement of DOC is relevant because:

- a high concentration of DOC in groundwater may reflect a significant surface water source indicating need for treatment if the water is used for drinking; and,
- OC concentrations influence the kinds and direction of redox reactions that determine concentrations of chemical species affecting the natural quality of

groundwater as well as the fate and transport of human-derived chemical contaminants that enter groundwater.

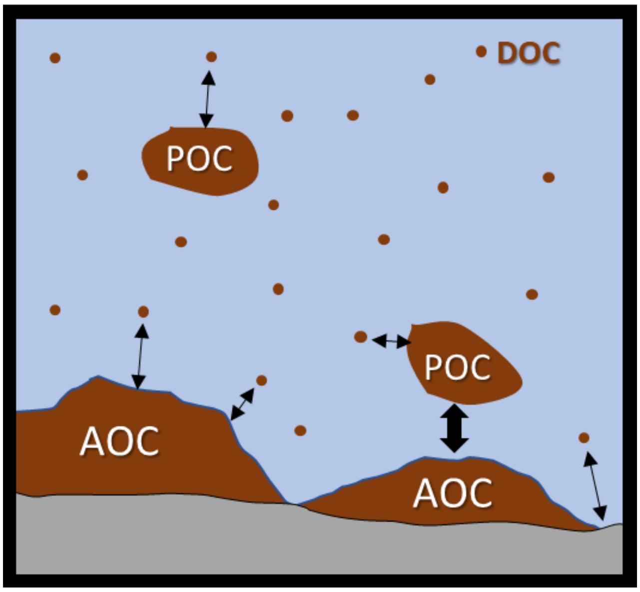


**Figure 4 -** AOC, POC and DOC near a grain or rock surface in an aquifer. Thin double-headed arrows represent adsorption and desorption of DOC to POC, AOC or to mineral surface. Heavier arrow represents adsorption and desorption of POC to AOC as discussed in the text. Although not illustrated in this figure, biodegradation processes discussed in later sections can convert AOC, POC and/or DOC to metabolites (products that result from breaking down the organic carbon), including dissolved inorganic carbon (DIC) (Mackay, 2022).

# 2 Historical Overview

## 2.1 Improved Analytical Methods Provided Insight into the Occurrence and Behavior of DOC

In 1970, a team of four U.S. Geological Survey hydrologists led by Jerry A. Leenheer began sampling wells throughout the United States and analyzing the groundwater for concentrations of DOC. Up to that time, there had been relatively few attempts to analyze groundwaters for DOC, primarily because it was difficult to measure the low concentrations that were typically encountered. Leenheer's team (R.L Malcolm, P.W McKinley, and L.A. Eccles) developed a method for the "wet oxidation" of filtered groundwater samples using sodium persulfate as an oxidant in a pressurized vessel at 175 °C followed by acidification and quantification of the carbon dioxide produced with an infrared analyzer, as illustrated in Figure 5. As improvement of analytical methods allowed detection of much lower concentrations of dissolved organic carbon, better field observations and laboratory experiments were conducted so, the occurrence and behavior of DOC was better understood.

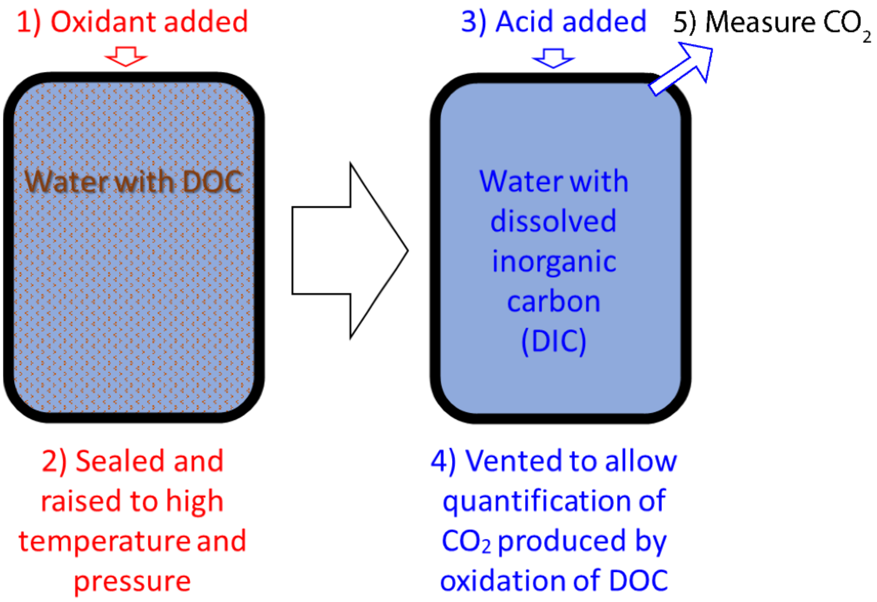


**Figure 5 -** The steps in quantifying low levels of DOC in water as described in the text. The DOC is oxidized to dissolved inorganic carbon (DIC), then the DIC is converted to dissolved $CO_2$ by acidification and the $CO_2$ is vented to a detector capable of quantifying total $CO_2$ evolved (Mackay, 2022).

This analytical methodology provided a lower detection limit for DOC of 0.1 milligrams per liter (mg/L), which is 8.3 micromoles per liter ($\mu$mol/L). The team collected and analyzed 100 groundwater samples from 27 states within the USA and from aquifers of differing lithologies that included sandstones, limestones, crystalline rocks, as well as shallow (< 200 feet or < ~60 m), and deep sand and gravel (> 200 feet or > ~60 m).

Their working hypotheses included the possibility that different aquifer lithologies and different sample depths might exhibit DOC concentrations.

Based on that study, Leenheer and others (1974) made four principal observations:

1) DOC concentrations in groundwater were generally much lower than commonly found in surface waters, with median concentrations ranging between 0.5 and 0.7 mg/L (~40 to ~60 $\mu$mol/L);
2) a shallow aquifer in Florida receiving active recharge from surface-water sources had much higher DOC concentrations (15 mg/L; ~1,250 $\mu$mol/L) than wells tapping the deeper Floridan aquifer (1.4 to 0.1 mg/L; ~120 to ~8 $\mu$mol/L) that was not immediately influenced by surface water;
3) there were no statistically significant differences in DOC concentrations between aquifers of different lithology; and,
4) there were clear statistical correlations between DOC concentrations, specific conductance (the ability of the water to conduct electricity, which increases with increasing concentration of dissolved ions), and alkalinity (the ability of the water to resist acidification, generally related to the content of bicarbonate ion, $HCO_3^-$, in water with near neutral pH).

Leenheer and others (1974) concluded that virtually all groundwaters contained low but measurable concentrations of DOC, that there were a variety of possible allochthonous (upgradient) and autochthonous (local) sources for DOC, and that DOC seemed to be involved in geochemical reactions with minerals present in aquifers that produced both dissolved solids and alkalinity. What was not clear was: the nature of those geochemical processes; how they affected the chemical composition of groundwater; and their hydrologic and ecologic significance.

Identifying those processes became the principal focus of groundwater DOC research in the years that followed. From the beginning, however, these studies had to overcome a fundamental difficulty. There are literally thousands of possible combinations for the organic carbon compounds present in DOC, even if consideration is limited to its three main elemental components carbon, hydrogen, and oxygen (Hertkorn et al., 2006). That, in turn, limits the utility of direct elemental analysis for characterizing the chemical and biological properties of DOC. For that reason, studies of both groundwater and surface water have been based on indirect analytical methods for characterizing the properties of DOC.

The first such indirect method was categorizing DOC based on its humic and fulvic acid composition, as illustrated in Figure 6 and discussed after the figure.

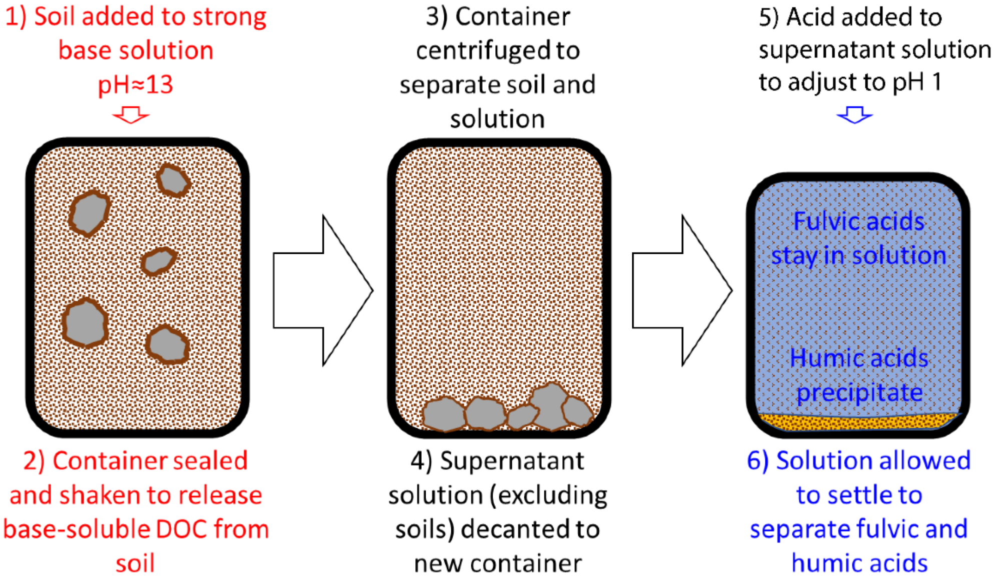


**Figure 6 -** Illustration of initial steps in characterizing soil organic matter for agricultural purposes. Humic and fulvic acids are extracted from soil by a strong base, then separated by acidification and settling. In the analysis of groundwater, this distinction is less useful since total DOC is generally low (Mackay, 2022).

Historically, characterizing organic matter in soils for agricultural purposes was based on extracting soils with strongly basic solutions (pH ~13) of sodium or potassium hydroxide (Achard, 1986). The resulting basic solutions were then acidified to a pH of 1 using hydrochloric acid, causing one fraction of the organic carbon, operationally defined as humic acids, to precipitate from solution. The fraction that did not precipitate was termed fulvic acids. Humic acids have a higher molecular weight (> 2000 unified atomic mass units, AMU) and are less water-soluble whereas fulvic acids have a lower molecular weight (800 to 2000 AMU) and are more water-soluble. While of historical interest, the humic-fulvic acid dichotomy is less useful for DOC present in groundwater, primarily due to the typically low concentration of DOC in groundwater (Thurman, 1985).

In a study of DOC concentrations in soil water, surface water, and groundwater of forested watersheds of the Adirondack Mountains, Cronan and Aiken (1985) showed that soil water collected in lysimeters from the *A* soil horizon (10 cm depth) had DOC concentrations of 21 to 32 mg/L (~1700 to ~2600 $\mu$mol/L), 5 to 7 mg/L (~400 to ~600 $\mu$mol/L) in soil water from the *B* soil horizon, and 2 to 4 mg/L (~170 to ~330 $\mu$mol/L) in shallow groundwater. They also found that the DOC consisted of both hydrophobic and hydrophilic fractions. This study clearly showed that DOC produced at the land surface from organic detritus had initially high concentrations and that those concentrations decreased markedly with depth. This study also hypothesized, as illustrated in Figure 7, that adsorption and microbial degradation processes were the major processes leading to the observed DOC removal.

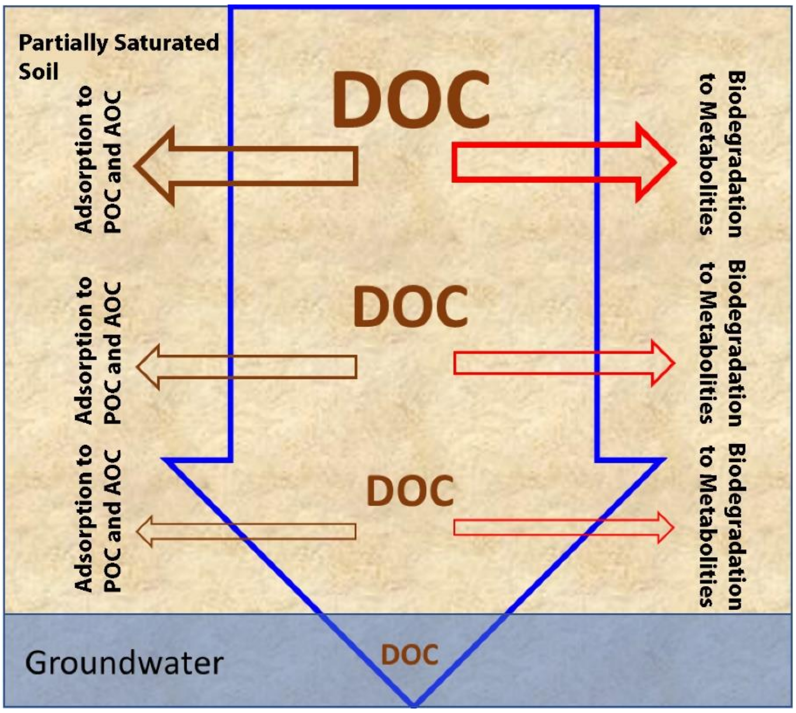


**Figure 7 –** DOC concentration decreases as water infiltrates from land surface to underlying groundwater due to adsorption to POC and AOC as well as biodegradation to metabolites. Mechanisms that decrease DOC concentrations as water infiltrates are: adsorption of DOC to POC, AOC and mineral surfaces; and, biodegradation to metabolites, including DIC. The weights of the arrows illustrate that adsorption may be less significant with depth because the remaining DOC is more hydrophilic with depth, and biodegradation may decrease with depth as the remaining DOC becomes less bioavailable (more resistant to biodegradation) (Mackay, 2022).

This adsorption/biodegradation hypothesis for DOC removal in groundwater was substantiated by later studies. Qualls and Haines (1992) observed that as much as 95 percent of DOC leached from leaf litter was removed as the water passed through the underlying unsaturated zone. Furthermore, the relative biodegradability of the remaining DOC was also observed to decline with depth once the water reached the underlying saturated zone. That suggested that some of the observed DOC removals were due to biodegradation processes. However, incubation experiments performed in that same study removed only about 33 percent of the DOC over 134 days. That, in turn, suggested that chemical adsorption onto mineral surfaces was also an important process contributing to the observed loss of DOC, a suggestion that was also observed by Davis (1982), Baham and Sposito (1994), and Lilienfein and others (2004).

An experimental approach examining DOC adsorption from water by soils was undertaken by Jardine and others (1989). Batch experiments were performed using water collected from a surface-water stream draining a peat deposit with an initial DOC concentration of 53 mg/L (4,400 $\mu$mol/L). Upon addition to soils, it was found that DOC concentrations decreased rapidly, coming to an equilibrium concentration in two days. The amount of DOC loss from solution at equilibrium was a function of initial DOC concentration, pH, and the mineral content of the soil. The soils used in the experiments contained significant amounts of POC and ferric oxyhydroxides coating the soil mineral grains. When much of the POC was removed from the soils by chemical extraction, the amount of DOC adsorption decreased by factors ranging from 5 to 10, demonstrating that POC itself was an important adsorbing substrate for DOC. Similarly, if the ferric hydroxides were removed by chemical extraction, the amount of DOC adsorption decreased by factors ranging from 2 to 3, indicating that ferric hydroxides were also a major adsorbing substrate. Furthermore, Davis (1982) had previously shown that clay minerals such as kaolinite were also a significant adsorbing substrate for DOC. The maximum amount of DOC adsorption in the study by Jardine and others (1989) was observed at a pH of 4.5, and adsorption was unaffected by increasing the ionic strength of the solutions.

This experimental study (Jardine et al., 1989) was consistent with the field results of Cronan and Aiken (1985) that showed rapid DOC removal as water moved through the soil zone to the water table. Furthermore, the study of Jardine and others (1989) suggested that increasing amounts of particulate organic matter and ferric oxyhydroxides in the soil increased the observed DOC adsorption. Finally, Jardine and others (1989) showed that hydrophobic DOC was more efficiently adsorbed (~80 percent) during the 2-day equilibration time as compared to hydrophilic DOC (~20 percent). That is consistent with the intuitive expectation that adsorption efficiency depends upon the specific chemical properties of each DOC component, as was suggested by Aiken (1989). Later, Jardine and others (2006) described how these processes affected the transport of DOC through soils at a variety of spatial scales.

The issue of DOC bioavailability (the proportion of DOC that can be readily biodegraded) in groundwater systems was initially investigated in the context of carbon flow and the ecology of stream ecosystems (Hornberger et al., 1994; Findlay and Sobczak, 1996; Boyer et al., 1997; Baker et al., 2000). These studies showed that DOC transported to streams by shallow groundwater flow supported populations of heterotrophic bacteria. Those bacteria, in turn, formed the lower trophic levels of stream ecosystems. While the focus of these studies was primarily ecological, they also provided direct evidence of the dynamic interactions between the DOC, POC, and AOC compartments. For example, these studies revealed marked annual cycles of rapid DOC delivery to streams during spring snowmelt "flush" followed by periods of less DOC delivery during the dryer parts of the year (Hornberger et al., 1994; Boyer et al., 1997; Baker et al., 2000). That implied for much of the year, DOC was actively being produced by biodegradation of plant detritus at land surface but was largely sequestered in soils, in part by adsorption processes. During the spring "flush", when snowmelt provided increased recharge to the shallow groundwater systems, DOC was mobilized from AOC and delivered to stream ecosystems. While not the stated purpose of these studies, these data showed that DOC adsorption could be a reversible process and that the DOC, POC, and AOC compartments were interconnected at some level.

During the 1990s, the analytical techniques available for studying groundwater DOC improved steadily. Grøn and others (1996) used a variety of analytical techniques including light absorbance, molecular weight distribution, $^{13}$C-NMR (13-carbon nuclear magnetic resonance) spectroscopy, elemental composition, and measurements of hydrolyzable amino acids and carbohydrates to characterize DOC in groundwater from three different aquifers in Denmark. Many of these analytical approaches had previously been developed for application to DOC in ocean water (Ogawa et al., 2001, Hertkorn et al., 2006, Sleighter and Hatcher, 2008) and river water (Hedges et al., 1994). Volk and others (1997) observed that 75 percent of the DOC present in Pennsylvania stream water, with concentrations ranging from 0.8 to 10.4 mg/L (67 to 866 $\mu$mol/L), was composed of humic substances. In that study, humic substances were measured by macroreticular XAD-resin chromatography (Thurman, 1985) defined as the difference between DOC concentrations prior to acidification and the effluent from the XAD-8 resin. It was also found that carbohydrates composed 13 percent and amino acids 2 percent of the DOC and were predominantly bound to humic acids.

The issue of DOC bioavailability began to attract wider attention in the 1990s. There already was a large literature devoted to assessing DOC bioavailability in the context of drinking water treatment technology (Servais et al., 1989). In addition, because the world's oceans contain much of the DOC present on earth, chemical oceanographers extensively investigated the issue of DOC bioavailability (Dauwe et al., 1999; Amon et al. 2001; Benner, 2003; Davis and Benner, 2007). One of the observations made by chemical oceanographers was that not all the organic matter present in the oceans is carbon and that other elements, notably oxygen, nitrogen, and phosphorous are also present. So, beginning in about the year 2000, the term "DOC" in the oceanographic literature was replaced by the more general term "dissolved organic matter" (DOM). In groundwater studies, however, the term DOC continued to be used (Meredith et al., 2019), and is generally considered as being synonymous with DOM (though strictly speaking DOM is about 50 percent DOC, as discussed earlier).

Kalbitz and others (2003) evaluated the bioavailability of soil-derived DOC using laboratory incubations that measured DOC loss, carbon dioxide ($CO_2$) production, changes in ultraviolet absorbance as well as emission fluorescence over time, and NMR spectroscopy before and after incubation. Hartog and others (2004) used incubation experiments measuring the consumption of dissolved oxygen over time as an indicator of aquifer sediment organic carbon bioavailability. Chapelle and others (2008) used cell counts, the ratio of DOC to total nitrogen, total hydrolyzable neutral sugars, total hydrolyzable amino acids, and *dissolved inorganic carbon* (DIC) production to compare the bioavailability of DOC present in a shallow sandy aquifer and a fractured-rock aquifer.

Beginning with the study of Leenheer and others (1974), the body of research discussed in this section leads to the view that there are three main compartments of organic carbon, DOC, POC, and AOC present in groundwater systems. Furthermore, extensive experimental evidence (Davis, 1982; Jardine et al., 1989) and field evidence (Qualls and Haines,1992; Baker et al., 2000; Jardine et al., 2006; Shen et al., 2015) indicate these compartments are not static but actively interact with each other.

## 2.2 DOC Fate Summarized in a Regional Chromatography Model

Based on these observed interactions, Aiken (1989) suggested that groundwater systems behave much like chromatographic columns which separate the components of DOC according to their atomic weights, their hydrophobicity, and their affinity for mineral grain surfaces. In Aiken's own words:

> *"Application of chromatographic theory to subsurface transport can aid in understanding and quantifying the chemical processes in subsurface systems. Chromatography is essentially the transport of a chemical in a mobile fluid phase through a column packed with a stationary phase. A chemical introduced at the beginning of this column moves at a rate proportional to the average velocity of the fluid and inversely proportional to the strength and nature of sorptive interactions with the stationary phase. These interactions include ionic and complexation interactions, hydrogen bonding, van der Waal's interactions, and equilibrium partitioning. In a ground-water system, the geologic matrix, representing the stationary phase, generally consists of sand grains coated with organic matter. The mobile phase is the water, which can contain significant quantities of dissolved organic and inorganic chemicals. Organic compounds move through the system as a result of the flow rate of water and the strength and nature of interactions with the stationary phase. The nature and distribution of organic matter in the system is determined, to a large extent, by the interactions between the various phases in the environment (emphasis added)."*

Shen and others (2015) came to similar conclusions in a study of DOC being delivered from forest floor leaf litter to a fractured-rock aquifer in the piedmont of South Carolina. In that paper, Shen and others (2015) proposed what they termed a "regional chromatography model" to illustrate the DOC dynamics that they observed (Figure 8).

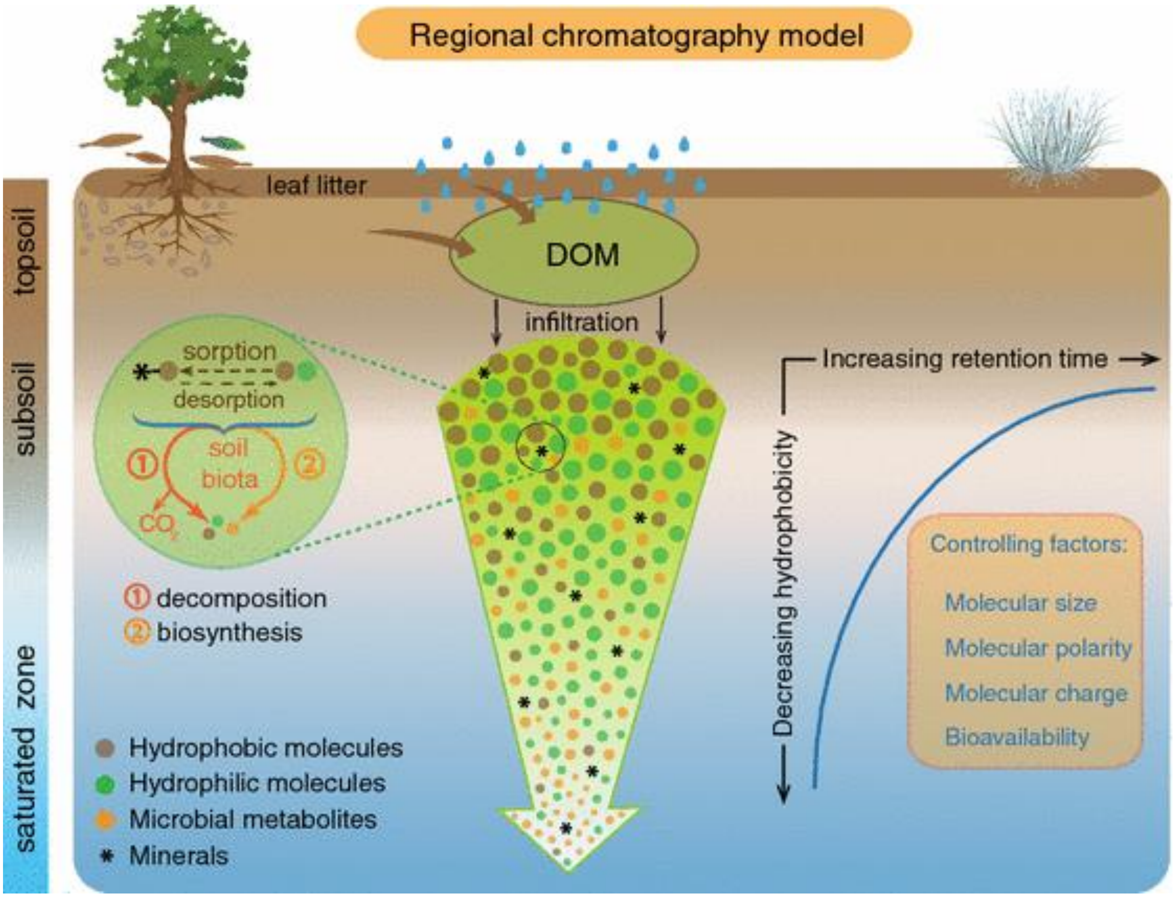


**Figure 8 –** Regional Chromatography Model - precipitation and surface water leaches dissolved organic matter (DOM) from vegetation and plant litter and percolates through the soil column to the saturated zone. The concentration, composition, and bioavailability of DOC are altered during transport through the soil column by various physicochemical and biological processes, including sorption, desorption, biodegradation, and biosynthesis. Hydrophobic molecules are preferentially partitioned onto soil minerals and have a longer retention time in soils than hydrophilic molecules. The hydrophobicity and retention time of colloids and dissolved molecules in soils are controlled by their size, polarity, charge, and bioavailability. Bioavailable DOC is subjected to microbial decomposition, resulting in a reduction in size and molecular weight. Novel molecules are synthesized by soil microbes, and some of these metabolites enter the DOC reservoir in groundwater. Reprinted with permission from Shen and others (2015).

# 3 Sources of Dissolved Organic Carbon to Groundwater Systems

There are three principal sources of DOC to groundwater. The first are allochthonous sources derived from plant material at land surface and transported to aquifers by infiltrating recharge water (Cronan and Aiken, 1985). Allochthonous DOC is typically the major source of DOC to shallow water table aquifers. The second major source is autochthonous DOC derived from particulate organic carbon that was buried with aquifer sediments at the time of deposition. Autochthonous DOC is typically a more important source of DOC to deeper confined aquifers (McMahon and Chapelle, 1991a). Finally, there are anthropogenic sources (carbon derived from human activities) of DOC as well. These anthropogenic sources include chemical contaminants such as petroleum hydrocarbons or chlorinated solvents (Wiedemeier et al., 1999) and DOC derived from agricultural activities (Thayalakumaran et al., 2015) and animal husbandry practices (Coote and Hore, 1979).

## 3.1 Allochthonous Sources of DOC

One of the earliest studies demonstrating awareness that plant material derived from land surface could be an important source of DOC to groundwater was that of Cronan and Aiken (1985). They showed that DOC produced at land surface from organic detritus had initially high concentrations and that those concentrations decreased rapidly with depth in the soil zone and shallow groundwater. That result was replicated in numerous later studies (Hornberger et al., 1994; Findlay and Sobczak, 1996; Boyer et al., 1997; and Baker et al., 2000). Those studies also showed that DOC delivered to streams by shallow groundwater was ecologically important, providing a carbon source for bacteria that formed the base of stream ecosystems. A generalized model for the delivery, sequestration, and remobilization of allochthonous DOC originating at land surface to groundwater systems is shown in Figure 8.

## 3.2 Autochthonous Sources of DOC

The model of allochthonous sources of DOC shown in Figure 8 predicts that both the concentration and bioavailability of DOC being delivered to an aquifer should decline as groundwater moves along the hydraulic gradient. Logically, therefore, if the sole sources of DOC to groundwater are allochthonous, then at some point concentrations of DOC should decline below measurable levels (~0.1 mg/L; ~8 $\mu$mol/L). That, however, is not what is observed. Measurable DOC concentrations are observed in groundwaters that are thousands or even millions of years old (time since recharge) which have moved hundreds of kilometers along aquifer flow paths (Leenheer et al., 1974). The reason for this observed behavior is that, in addition to allochthonous sources of DOC, there are many potential sources of autochthonous DOC in groundwater systems as well. In addition to delivering a steady supply of DOC to groundwater, autochthonous organic carbon in subsurface environments is also the source of petroleum and natural gas (Espitalie et al., 1980). Whereas the origin of petroleum and natural gas has been subject to active study for many years, the origin of autochthonous DOC in groundwater system used for water supply has only recently been investigated.

Initially, the motivation for investigations into autochthonous sources of DOC was the puzzling observation that, while concentrations of DOC in deep confined aquifers did not seem to change significantly along aquifer flow paths, concentrations of dissolved inorganic carbon (DIC) increased (McMahon and Chapelle, 1991b). Because the groundwater in that study was saturated with respect to calcite, it seemed unlikely that the increase in DIC could come exclusively from calcite dissolution. An alternative explanation was that there was a source of organic carbon that, when oxidized, could provide the observed DIC. While little organic carbon was present in sandy aquifer material in that system, there was abundant organic carbon in the clayey beds that confined the aquifers. Cores were recovered from both the aquifer and confining beds and their pore water was analyzed for the presence of organic acids. The results showed that while concentrations of formate and acetate were low in the pore water of sandy aquifer sediments, concentrations were much higher in the pore water of clayey confining beds. Furthermore, diffusion of the organic acids from the clayey beds to the sandy aquifers, as illustrated in Figure 9, could transport the DOC to the aquifer, which upon oxidation could provide a source of dissolved inorganic carbon (McMahon and Chapelle, 1991a). A later study (Chapelle and Bradley, 1996) demonstrated that the presence of organic acids in clayey sediments was associated with active microbial acetogenesis (production of acetate).

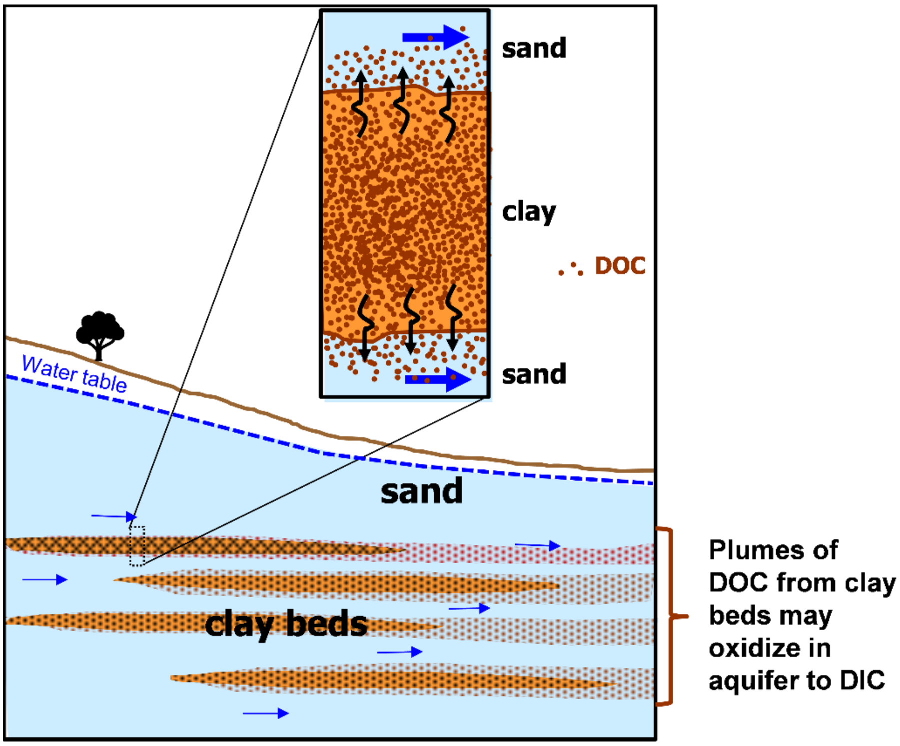


**Figure 9 –** Low permeability geologic units with almost no active flow (e.g., clay beds) can release old DOC by diffusion (brown dots in inset or shading) to the active flow in the permeable zones (e.g., sands), so the groundwater flowing around the clay beds may contain DOC from the clay beds that may oxidize to DIC along the flow path in the permeable zone. Only a few large clay beds are shown for clarity (Mackay, 2022).

The production and diffusion of DOC from confining beds to aquifers has been observed in other deep confined aquifer systems (Lawrence et al., 2000, Hendry and Wassenaar, 2005). Figure 10 shows concentrations of formate, acetate, and propionate in pore water derived from deep sediments (810 to 890 m) of the Atlantic Coastal Plain underlying Hilton Head Island, South Carolina (Chapelle and Bradley, 1996). Note the millimolar concentrations of organic acids associated with pore water from the clayey sediments and their near absence in pore water from the sandy sediments. These sediments are of Upper Cretaceous age (~80 MY) demonstrating that organic carbon capable of supporting microbial organic acid production can persist for tens of millions of years in low-permeability clayey sediments.

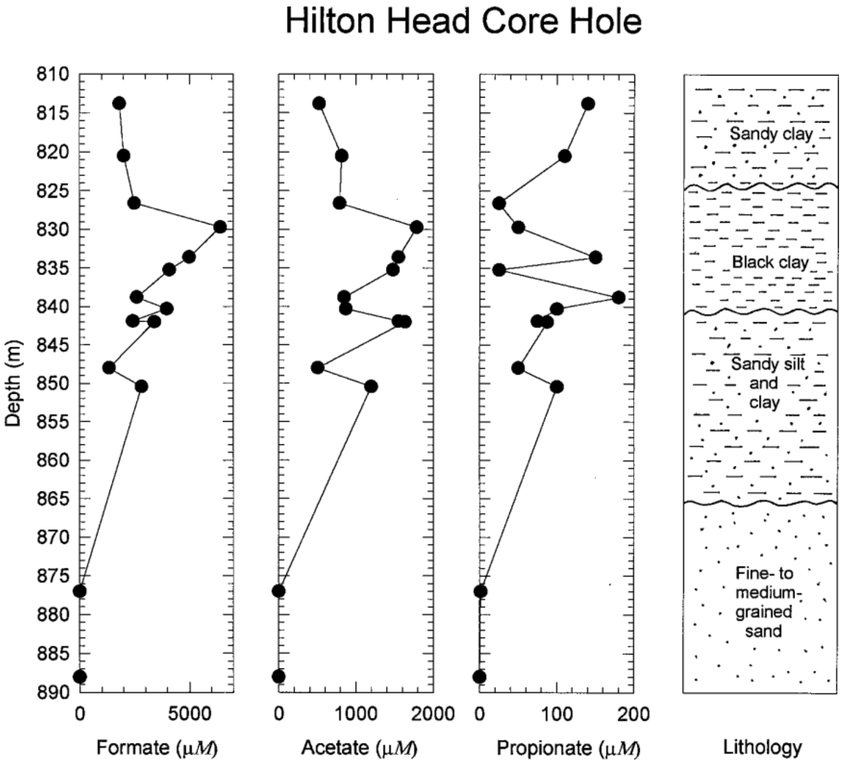


**Figure 10 –** Data from the Hilton Head core hole showing the relationship between concentrations of dissolved formate, acetate, and propionate in pore water of the permeable zones and confining bed within the Black Creek aquifer system (reprinted from Chapelle and Bradley, 1996, with permission).

## 3.3 Anthropogenic Sources of DOC

One of the defining characteristics of groundwater in pristine aquifer systems (those that have not been chemically affected by human activities) is that DOC concentrations are relatively low, in the 0.4 to 4.0 mg/L (~30 to ~330 $\mu$mol/L) range (Regan et al., 2017). In contrast, groundwater that has been chemically affected by human activities frequently exhibits DOC concentrations in the 4.0 to 120 mg/L (~330 to ~10,000 $\mu$mol/L) range (Regen et al., 2017). Because of that observation, DOC concentrations greater than 4.0 mg/L (~330 $\mu$mol/L) have historically been used as a qualitative indicator of chemical contamination by human activity (Barcelona, 1984). A variety of human activities including sewage disposal, tilled agriculture, feedlot operations, petroleum hydrocarbon storage facilities, and industrial solvent disposal have the potential for delivering dissolved organic compounds to groundwater.

### 3.3.1 Tilled Agriculture

An example of tilled agriculture affecting DOC concentrations in groundwater was described by Thayalakumaran and others (2015). The study site is in Queensland, Australia, in some of that country's most productive irrigated farmland. In particular, the area is noted for some of the highest yields and highest quality of sugarcane in Australia. The agricultural land is underlain by a sandy aquifer of alluvial and marginal marine origin.

The tilled farmland is heavily irrigated with water derived from wells and the nearby Burdekin River. The combination of the principal crop (sugarcane) and rapid recharge of percolated irrigation water produces an abundance of both particulate and dissolved organic carbon. The resulting DOC provides significant reducing potential that has had a dramatic effect on the groundwater chemistry of the shallow aquifer. Concentrations of DOC range from 5 to 50 mg/L (~40 to ~410 $\mu$mol/L), concentrations of dissolved oxygen and nitrate are generally less than 1 mg/L, and concentrations of dissolved iron often exceed 1 mg/L. These reducing conditions have both positive and negative effects on groundwater quality. On the positive side, the high concentration of bioavailable DOC produces anoxic conditions that foster nitrate reduction, effectively preventing excessive concentrations of nitrate from accumulating in the groundwater. On the negative side, those conditions also promote Fe(III) reduction leading to high concentrations of dissolved iron that can lead to problems such as clogging of irrigation wells.

### 3.3.2 Feedlot Operations

An example of cattle feedlot operations affecting DOC concentrations and groundwater chemistry was reported by Coote and Hore (1979). The study site was in Ontario, Canada, and had been used as a feedlot for 6 years. Before the feedlot operation, the site had been a barnyard for the previous 80 years. The feedlot was approximately 24 m × 34 m in size and was underlain by a shallow sandy aquifer with the water table approximately 2 m below land surface. A system of shallow, depth-nested sampling wells was installed adjacent to the feedlot extending 130 m downgradient. Not surprisingly, DOC concentrations just two meters downgradient of the feedlot and just below the water table were high (~700 mg/L; ~60 mmol/L), and DOC concentrations 130 m downgradient decreased only to 79 mg/L (6.5 mmol/L). Chloride concentrations along that same flow path decreased from 664 mg/L to 79 mg/L, virtually identical to the change in DOC concentrations. That, in turn, suggests that the concentration decreases reflect mixing with uncontaminated recharge water and that biodegradation was less important. However, the high levels of DOC near the feedlot show that feedlots can be a significant source of DOC to groundwater.

### 3.3.3 Petroleum Hydrocarbons

An example of petroleum hydrocarbons affecting concentrations of DOC in groundwater was described by Petkewich and others (1997). The study site was a jet fuel storage facility in Hanahan, South Carolina where an estimated 83,000 gallons (~314,000 L) of Jet Propellant-4 fuel leaked from a tank in 1975 (Vroblesky et al., 1997). A plume of petroleum hydrocarbon-contaminated groundwater developed over time and migrated beneath an adjacent housing development. A series of strategies were employed to remediate the site, and in 1990 a pump-and-treat system was installed to collect contaminated water. For the next four years, groundwater chemistry from each of 18 extraction wells was monitored for a variety of analytes, including DOC and total petroleum hydrocarbons (TPH). Measurements of DOC would include the contribution of TPH compounds. However, concentrations of TPH compounds would not reflect the influence of naturally occurring DOC generated from plant material at land surface.

Concentrations of DOC and TPH hydrocarbons measured in groundwater collected from one of the extraction wells (EW-8) are shown over a four-year period in Figure 11. Initially, groundwater produced from the well had relatively low DOC concentrations (3.0 mg/L; 250 µmol/L) and TPH concentrations below the detection limit (11 mg/L; ~85 µmol/L). This reflects the fact that well EW-8 was located outside the existing plume of contaminated groundwater. After initiation of pumping, however, petroleum hydrocarbon-contaminated water was drawn to the well and concentrations of both TPH and DOC initially increased (Figure 11). Over time, however, DOC concentrations continued to increase whereas TPH concentrations remained static at about 1.5 mg/L (125 µmol/L). By the end of the monitoring period (1996), DOC concentrations had more than doubled from their initial levels (~8 mg/L; ~670 µmol/L) whereas TPH concentrations were once again near or below the detection level (1 mg/L).

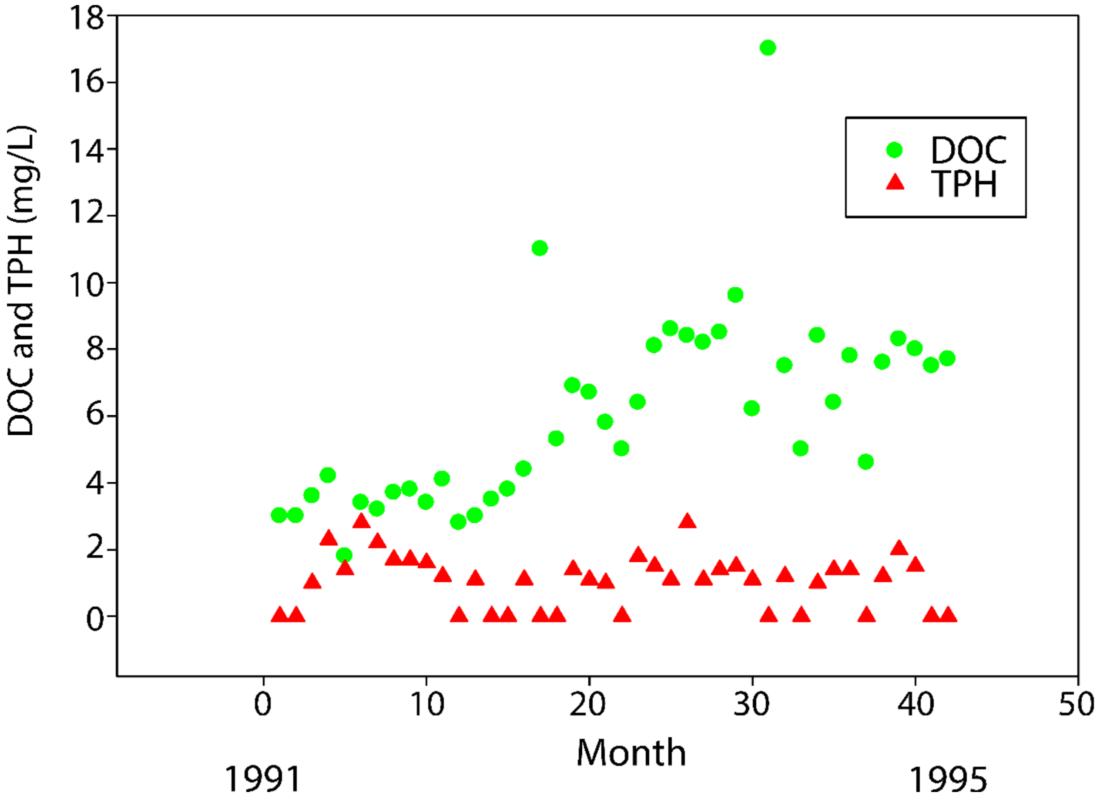


**Figure 11 -** Concentrations of dissolved organic carbon (DOC) and total petroleum hydrocarbons (TPH) measured in groundwater from extraction well EW-8 between 1991 and 1996 at the Hanahan SC, fuel storage facility. Data are from Petkewich and others (1997).

The data in Figure 11 show some interesting trends that are probably representative of processes occurring at other petroleum hydrocarbon-contaminated sites as well. The increasing DOC concentrations reflect, in part, drawing TPH to well EW-8 (Figure 11) as soon as pumping commenced. But the relatively low TPH concentrations (1 to 2 mg/L) sustained over time cannot account for the entire observed increase of DOC concentrations (to ~8 mg/L; ~670 $\mu$mol/L). The source of DOC present in the initially uncontaminated groundwater were plants growing at land surface. But as pumping drew TPH to the well, the availability of a new source of metabolizable carbon (TPH) seems to have stimulated TPH biodegradation. This new carbon source (TPH) may have stimulated microbial activity, increased production of microbial metabolites (Figure 8), and those metabolites subsequently increased overall DOC concentrations (Figure 11).

### 3.3.4 Chlorinated Solvents

Dissolved organic carbon that is either naturally occurring or is artificially added to groundwater, can serve as an electron donor driving the reductive biodegradation of chlorinated solvents in groundwater (Chapelle et al., 2012). An example of naturally occurring DOC driving reductive dechlorination (Bradley et al. 2009) with artificially added DOC enhancing the process was described by Bradley and others (2012). The site is a decommissioned Naval Air Warfare Center (NAWC) located in West Trenton, New Jersey that is underlain by fractured, Triassic-age mudstone shales and sandstones. Large quantities of trichloroethene (TCE) were used at this site as a refrigerant to simulate high-altitude temperatures for testing jet engines between 1956 and 1998. Over the years, accidental spills of TCE contaminated the underlying bedrock aquifer, and efforts to remediate that contamination were initiated in 1995 (Lacombe, 2000).

The NAWC site is one of only a few where concentrations of DOC and chlorinated ethenes were monitored under first naturally occurring conditions, and then after adding artificial DOC to further enhance biodegradation. Natural DOC at this site is generated in the soil zone overlying the bedrock and is transported to the fractured aquifer by percolating recharge. The artificial DOC added to enhance reductive dichlorination consisted of an emulsified vegetable oil solution (EOS$^{\text{TM}}$) that included sodium lactate (Borden et al. 2007). Some results of the NAWC study are shown in Figure 12.

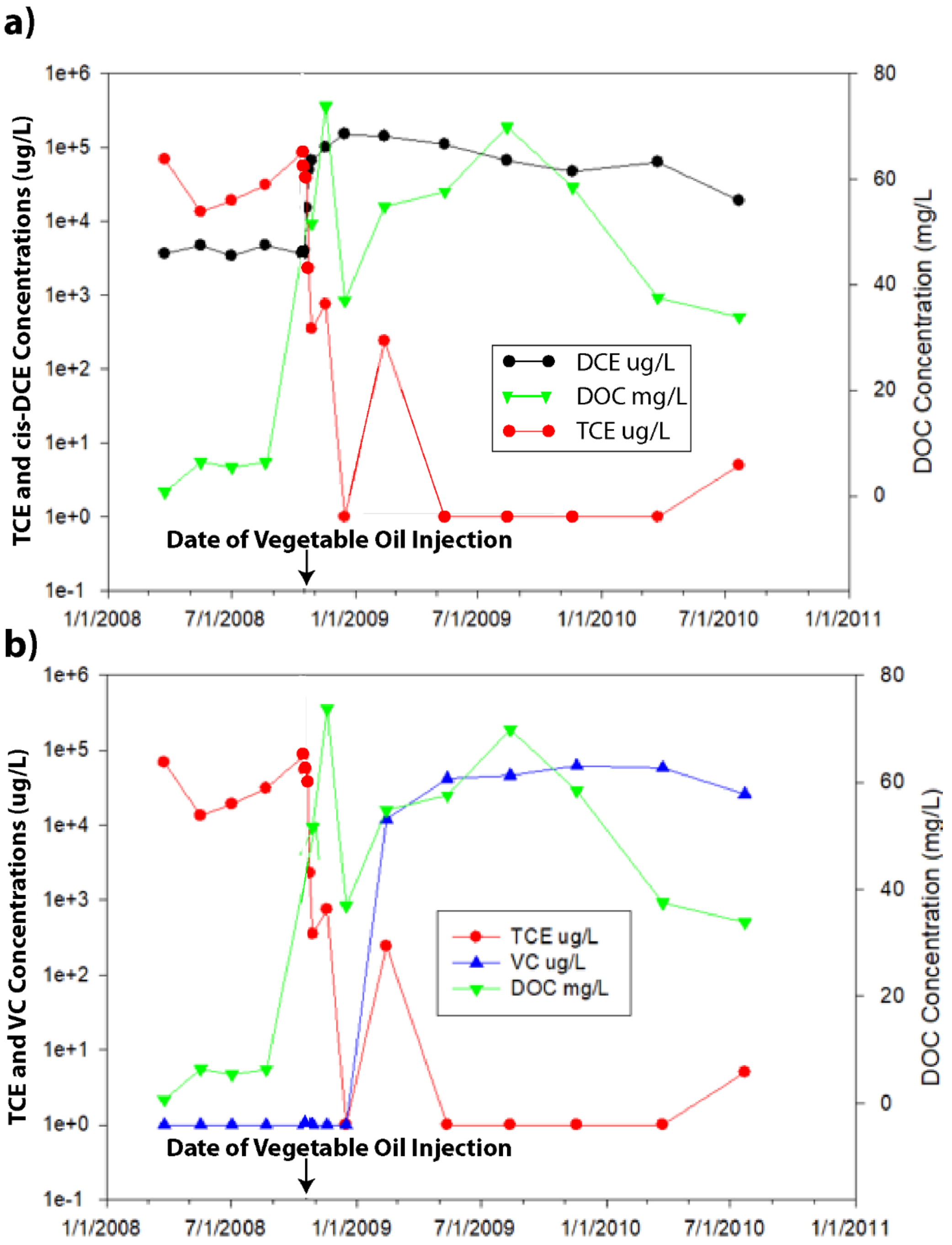


**Figure 12 -** Concentrations of a) TCE, cis-DCE, and DOC and b) TCE, VC, and DOC at the NAWC site in well BR-36 before and after bioremediation using emulsified vegetable oil.

Prior to October 2008, naturally occurring DOC generated in the soil zone was the sole electron donor supporting the degradation of the chlorinated ethenes. That natural DOC (~5 to 10 mg/L; ~400 to ~800 $\mu$mol/L) supported reductive dichlorination of TCE (~5,000 $\mu$g/L) as indicated by the high concentrations (~4,000 $\mu$g/L) of cis-dichloroethene (cis-DCE), the first degradation product of TCE (Figure 12a). Concentrations of vinyl chloride (VC), the degradation product of cis-DCE, however, were less than 20 $\mu$g/L. This suggested that natural DOC, while capable of driving reductive dichlorination of TCE, was less able to drive cis-DCE dichlorination. After addition of the emulsified vegetable oil/sodium lactate solution, the higher concentrations of DOC (~40 to 60 mg/L; ~3,300 to 5,000 $\mu$mol/L) led to increased concentrations of both cis-DCE (Figure 12a) and VC (Figure 12b) while lowering concentrations of TCE. These studies show that both natural and artificially added DOC can drive reductive dichlorination in chlorinated-ethene contaminated groundwater.

# 4 Organic Chemistry of DOC

A fundamental difficulty in understanding the chemical properties of naturally occurring DOC is that it is chemically complex. There are literally thousands of combinations of organic carbon moieties (organic molecules that can combine to form larger organic molecules) present in DOC, including quinones, phenols, catechols, amino acids, and sugars. Longnecker and Kujawinski (2011), for example, used an analytical technique called electrospray ionization coupled with Fourier-transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) to examine DOC found in groundwater. Figure 13 shows the thousands of peaks (individual moieties) found in a single groundwater sample of DOC, vividly illustrating its compositional complexity. This complexity, in turn, limits the utility of direct chemical analysis for understanding the properties of DOC. For that reason, studies of both groundwater and surface water have historically used indirect analytical methods for characterizing the properties of DOC.

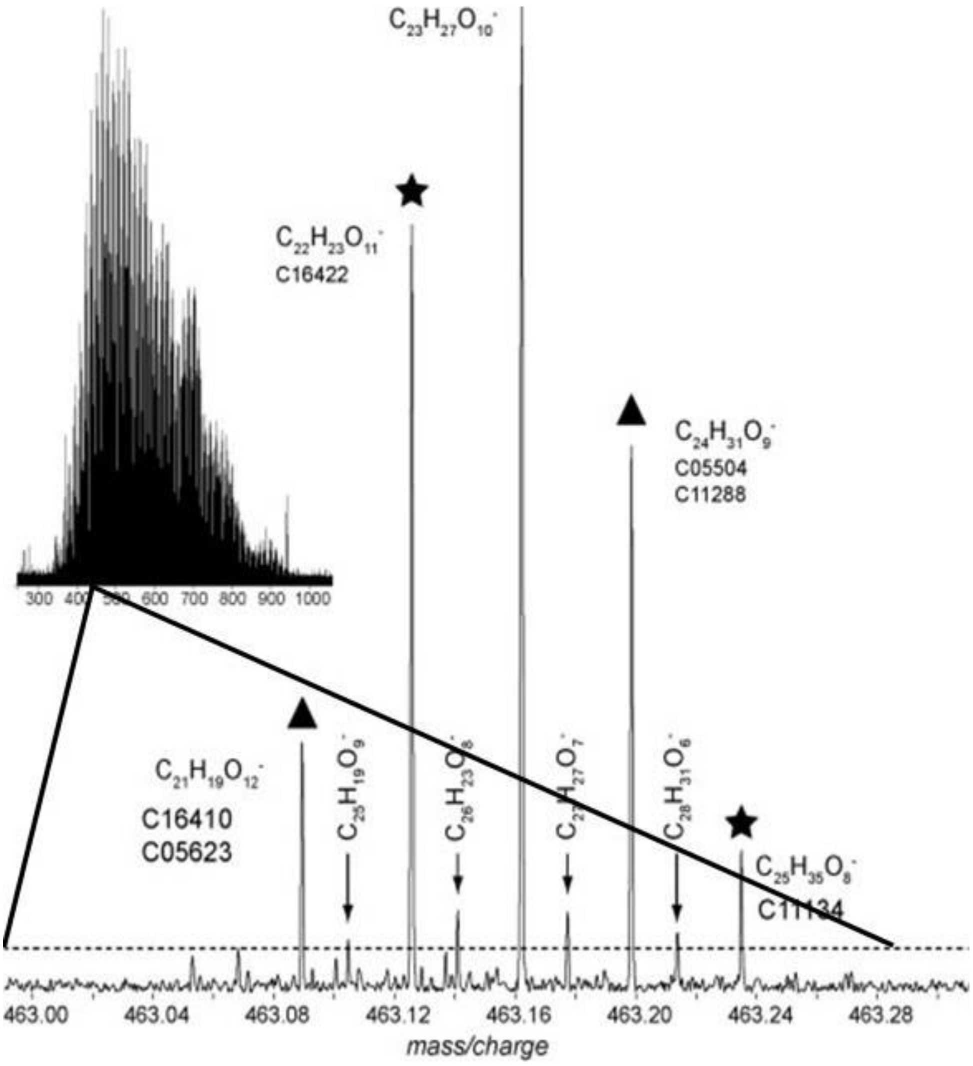


**Figure 13 -** Negative ion mode ESI FT-ICR MS mass spectra of DOC from whole groundwater illustrating its compositional complexity. Reproduced from Longnecker and Kujawinski (2011) with permission.

## 4.1 Humic and Fulvic Acids

The first such indirect method for characterizing DOC was based on its humic and fulvic acid composition as has been discussed previously. Artinger and others (2000) provided an example of how quantifying humic and fulvic acids can be used to characterize groundwater DOC. This study collected 35 different samples of groundwater from four different aquifer systems in Germany. Because of the low DOC concentrations encountered, it was necessary to concentrate the DOC by adsorption onto XAD-8 resins as described by Thurman (1985). In samples exhibiting the low DOC typically observed in groundwater (0.1 to 2 mg/L; ~8.3 to ~170 $\mu$mol/L), humic acids comprised a relatively low percentage of the total DOC (< 10 percent). Fulvic acids, on the other hand, comprised up to 80 percent of total DOC in the low-DOC groundwaters. That is consistent with the greater solubility of fulvic acids. In contrast, in groundwater containing higher concentrations of DOC (10 to 100 mg/L; ~830 to ~8,300 $\mu$mol/L), humic acids comprised a much larger percentage of the total DOC (> 50 percent). Similar results have been found in other groundwater systems. In general, higher amounts of humic acids are associated with higher overall DOC concentrations.

## 4.2 Hydrophilic, Hydrophobic, and Neutral Fractions of DOC

A more advanced procedure, derived from the humic/fulvic dichotomy, is termed "dissolved organic carbon fractionation analysis" (Leenheer and Huffman, 1979). In this method, DOC is first concentrated on XAD-8 resins and separated into hydrophobic and hydrophilic fractions by selective desorption using acidic and basic eluents. Those two fractions are then each further separated into acids, bases, and neutral fractions, giving a total of six fractions. This method of analysis was used to characterize the composition of soil water and groundwater in a forested watershed of the Adirondack Mountains in New York (Cronan and Aiken, 1985). Their results showed, for the first time, that groundwater DOC was dominated by hydrophilic and hydrophobic acids, with a lesser contribution from bases and neutral organic compounds. This fractionation technology has continued to evolve and is still used to characterize DOC in groundwater and surface-water systems (Chow et al., 2004; Ratpukdi et al., 2009).

Modifications of this fractionation methodology has been used to characterize DOC in a variety of aquifer systems. Hendry and Wassenaar (2005) described a study of DOC in confining-bed porewaters of a deep aquifer system in western Canada. They focused their study on the high-molecular weight (HMW) and low molecular weight (LMW) fractions of DOC and their respective carbon-13 and carbon-14 compositions. The results showed a systematic decrease in DOC concentrations and the HMW and LMW fractions with depth (42 m) in pore waters of the confining bed material. They also showed a systematic decrease in the $\delta^{13}$C and $^{14}$C activity with depth, suggesting the observed DOC was derived from soil organic carbon of Holocene (~10,000 years BP - before present) soils, and older (15,000 years BP) pore water.

## 4.3 Elemental Composition of DOC

Another method for characterizing DOC in groundwater (Artinger et al., 2000; Murphy et al., 1989) is by direct chemical analysis. Reported analyses of groundwater show that DOC is typically composed of carbon (50 to 58 percent), oxygen (33 to 42 percent), hydrogen (4.4 to 6 percent), sulfur (1.6 to 4.5 percent), and nitrogen (0.5 to 2 percent). The usefulness of elemental composition in understanding the sources and diagenetic history of DOC, however, is not always clear. Artinger and others (2000) compared differences in elemental composition between a young aerobic groundwater sampled from a quaternary-age aquifer of glacial origin (Munich) and an older anaerobic groundwater sampled from a Triassic-age sandstone aquifer (Franconian) in Germany. The DOC concentrations in Munich groundwater were somewhat higher (0.6 to 0.9 mg/L; 50 to

75 μmol/L) than Franconian groundwater (0.1 to 0.5 mg/L; ~8 to ~40 μmol/L). There were not, however, dramatic differences in elemental composition. The carbon content of DOC was slightly higher in Franconian DOC (56.3 ± 2.1 percent) relative to Munich DOC (52.2 percent ± 0.6 percent). Similarly, the hydrogen content of Franconian DOC (6.1 ± 0.5 percent) was slightly higher than Munich DOC (4.4 ± 0.2 percent), but it is unlikely those differences are statistically significant. The oxygen content of both Franconian and Munich DOC was virtually identical (~37 percent). Because of the differences in groundwater ages and aquifer types, those systems might have been expected to exhibit more differences in elemental composition. That, however, was not observed.

Murphy and others (1989), in a study of the Milk River aquifer system in Canada, showed a general decrease in DOC concentrations along the regional hydrologic gradient (from ~6 to 1.5 mg/L; i.e., 500 to 125 μmol/L). There was not a systematic change in DOC carbon content (~56 percent), or hydrogen content (~6.5 percent) between upgradient and downgradient groundwater. There was, however, a slight decrease in oxygen content between upgradient (~35 percent) and downgradient (~31 percent) DOC samples. In any case, the utility of using bulk elemental composition to characterize groundwater DOC remains an open question.

More recently and using more sophisticated analytical procedures for elemental analyses of DOC have shown further promise (McDonough et al., 2020). Using a combination of liquid chromatography-organic carbon detection (LC-ODC) and negative-ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) the Connected Waters Initiative Research Centre in Sydney, Australia has shown how the elemental character of DOC in a shallow coastal aquifer varies temporally and spatially depending on atmospheric precipitation events. Those variations, which have only recently come to light, are very likely to be observed in other groundwater systems.

## 4.4 Solid-state C-13 NMR

The stable isotope of carbon $^{13}$C has the interesting property that it has "nuclear spin", a property which is analogous to the spin of electrons. Carbon-12 ($^{12}$C), the most common isotope of carbon, lacks nuclear spin. Because of its nuclear spin, $^{13}$C atoms behave in a similar fashion to a tiny bar magnet whereas $^{12}$C atoms do not. In the absence of a magnetic field, the $^{13}$C atoms are randomly oriented, as are the $^{12}$C atoms. But when a magnetic field is applied, the $^{13}$C atoms line up parallel to that field, either spin-aligned or spin-opposed, while C-12 atoms are unaffected by the magnetic field.

If a sample of carbon is subjected to a magnetic field of increasing strength, at some point all the $^{13}$C atoms will align themselves to that field, a point known as *resonance*. However, because carbon atoms form chemical bonds with hydrogen, oxygen, nitrogen and other carbon atoms, the nature of those bonds determines when resonance is achieved for individual carbon atoms. Thus, by placing a sample of mixed carbon compounds in a magnetic field and recording the energy levels at which $^{13}C$ atoms attain resonance, it is possible to deduce some of the chemical properties of the carbon compounds present. That analytical technique is known as 13-C nuclear magnetic resonance or 13 Carbon-nuclear magnetic resonance. This is one of the analytical techniques that have been used to investigate the chemical properties of DOC in groundwater (Aiken et al., 1985; Grőn et al., 1996; Murphy et al., 1989; Aravena et al., 2004). While the underlying principle of NMR, with $^{13}C$ atoms acting as tiny magnets lining up with a magnetic field is easy to grasp, the physics behind exploiting those properties is quite complex. Furthermore, because non-magnetic $^{12}C$ accounts for 98.9 percent of all carbon atoms, NMR applies only to a small minority of the carbon present. That, in turn lowers the sensitivity of the method.

The NMR technique that has seen the most use for studying DOC in groundwater (Aiken et al., 1985; Grőn et al., 1996; Aravena et al., 2004) is known as solid-state cross polarization/magic angle spinning (CP/MAS). The first step in this procedure is to collect solid samples of carbon (hence the term "solid state"). This can be done by precipitating the humic and/or fulvic acid fractions of DOC at low pH with subsequent drying. It might take as much as a hundred liters of groundwater to provide enough humic or fulvic acids for solid-state NMR analysis. Next, these samples are packed tightly into rotors which are then spun at rates from 1 to 35 kHz in a spectrometer. Magic-angle spinning introduces artificial motion by placing the axis of the sample rotor at the magic angle ($54.74^{o}$) with respect to an external magnetic field. This technique provides a way to obtain quantitative solid-state $^{13}C$-NMR spectra of organic materials with an acceptable signal-to-noise ratio. As the magnetic field strength increases, the number of $^{13}C$ atoms reaching resonance is recorded on the y-axis as a spectrum. Because different $^{13}C$ functional groups achieve resonances at different field strengths, it is possible to estimate the relative abundance of those functional groups by quantifying the area under the spectral curve.

An example of how $^{13}C$-NMR has been used to characterize DOC in groundwater was provided by Aravena and others (2004). These researchers were studying the Alliston aquifer, a regional confined aquifer in southern Ontario, Canada. The aquifer is of glacial origin and is composed of sand and gravel lenses confined above by thick clay tills and below by Paleozoic bedrock. The groundwater chemistry of the Alliston aquifer is characterized by relatively high concentrations of DOC (~10 mg/L) and high concentrations of dissolved methane (~20 mg/L). These high DOC concentrations made it possible to extract sufficient solid-phase fulvic acids from approximately 100 liters of groundwater for solid-state $^{13}C$-NMR analysis.

Some of the results reported by Aravena and others (2004) are shown in Figure 14. These results indicate the relative abundance of carboxyl (COOH) groups, aromatic (carbon ring structures, and aliphatic carbon (carbon chains) present in the DOC. Note that the horizontal scale has a zero value. That zero point is where resonance is achieved by the 13-C atoms in tetramethylsilane (TMS) which serves as a standard to which the resonance characteristics of other compounds can be compared. In this study, the results indicate that the fulvic acid fraction of DOC is dominated by aliphatic carbon (~65 percent) with lesser amounts of aromatic carbon (~25 percent) and carboxyl carbon (~10 percent).

The relative proportion of aliphatic, aromatic, and carboxyl carbon in the fulvic acid fraction of DOC (Figure 14) seems to vary between different hydrologic systems. In a study of three different aquifers of marginal marine origin in Denmark (Grőn et al., 1996), aromatic carbon predominated (51 percent) in DOC from one aquifer (the Fjand aquifer). In contrast, DOC from two other aquifers (the Skagen and Tuse aquifers) was composed of predominantly aliphatic carbon (51 percent). These observed differences probably reflect the source materials (marine versus terrestrial) for the DOC as well as their diagenetic history.

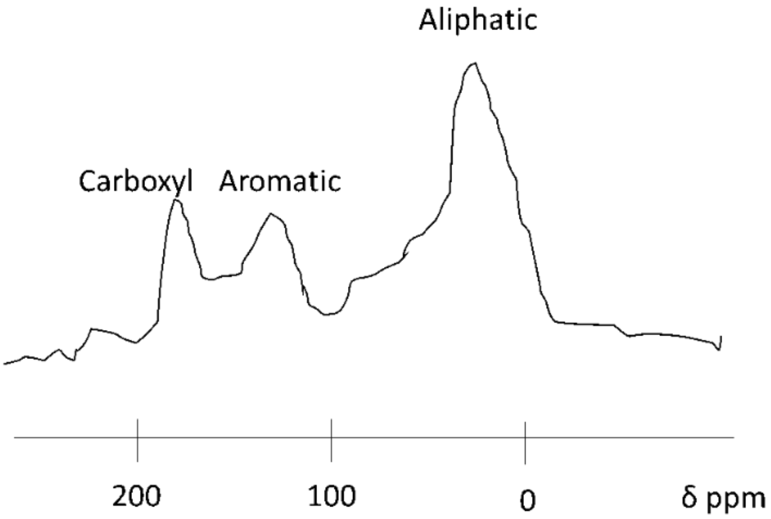


**Figure 14 -** An example of a $^{13}$C-NMR spectrum showing the approximate relative abundance of carboxyl, aromatic, and aliphatic carbon present in the fulvic acid fraction of DOC. Data are from Aravena and others (2004).

## 4.5 UV Absorbance

Much of the brownish color associated with some natural groundwater and surface water is due to the presence of DOC. Some DOC is capable of absorbing ultraviolet (UV) and visible (V) light thereby conferring the brownish color. This color-producing DOC is referred to as chromophoric or cDOC.

The absorbance of radiation by compounds such as cDOC depends on the compound's electronic structure. In the case of the near UV ($\gamma = 200\text{-}380$ nm), conjugated organic molecules (those with delocalized electrons such as are present in benzene) have the greatest UV absorbances. Because humic substances present in DOC are characterized by chains of alternating single and double-bonded aromatic carbon atoms, they have delocalized electrons and are able to absorb UV radiation. The absorbance of UV radiation by naturally occurring DOC is typically proportional to its concentration. For that reason, absorbance at 254 nm is often used in the water-treatment industry as a surrogate parameter for DOC concentrations. However, because the aromatic content of DOC can vary significantly between different groundwater systems, that approach is not typically useful in groundwater studies.

Variation of UV absorbance between hydrologic systems is illustrated by Figure 15 and Figure 16. Figure 15 shows the locations of eight different aquifer systems located throughout the United States (Chapelle et al., 2016). Samples from the South Carolina sites showed DOC concentrations that ranged from < 0.1 to 5.6 mg/L (< 8 to 430 $\mu$mol/L) and absorption coefficient values at 254 nm, $a_{\gamma\,254}$, ranging from zero to 420 m$^{-1}$. The calculation of $a_{\gamma\,254}$ from measured UV absorbance is given by Equation 1.

$$a_{\gamma 254} = 2.303\ A_{\gamma}/r \tag{1}$$

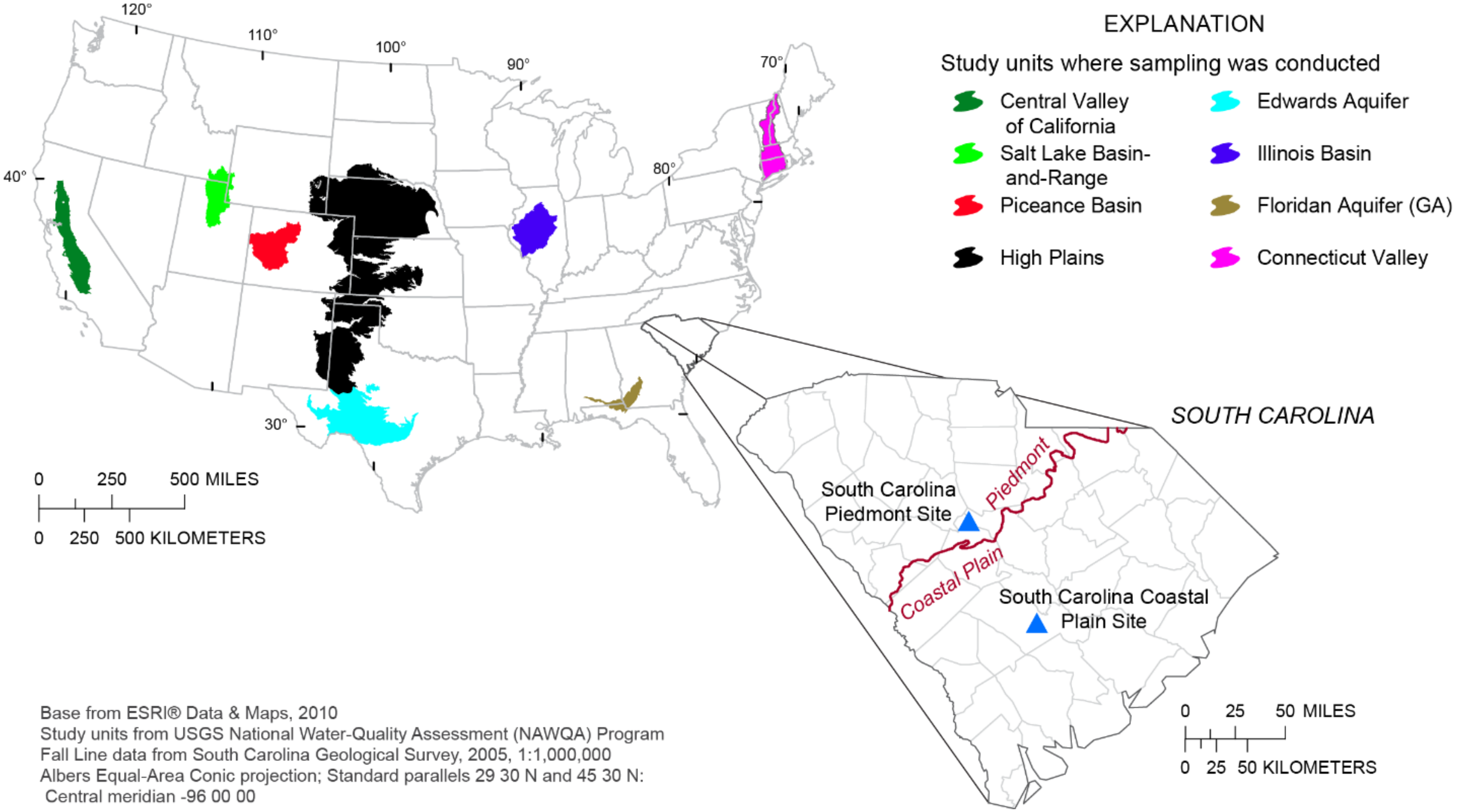
where:

$\alpha_{\gamma}$ = absorption coefficient (L$^{-1}$)

$A_{\gamma}$ = measured UV absorbance at 254 nm (dimensionless)

$r$ = Path length (L)

Use of absorption coefficients reflects the fact that the low absorbances typical of most groundwaters are measured using a 10 cm pathlength cuvette whereas higher absorbance samples are measured using a 1 cm pathlength cuvette. The South Carolina samples were used to delineate how DOC concentrations varied relative to $a_{\gamma\,254}$ and that "DOC/$a_{\gamma\,254}$ path way". That path way was compared to DOC concentrations and $a_{\gamma\,254}$ values from seven other aquifer systems in the United States. The results of this comparison are shown in Figure 16.



Base from ESRI® Data & Maps, 2010
Study units from USGS National Water-Quality Assessment (NAWQA) Program
Fall Line data from South Carolina Geological Survey, 2005, 1:1,000,000
Albers Equal-Area Conic projection; Standard parallels 29 30 N and 45 30 N:
Central meridian -96 00 00

**Figure 15 -** Locations of the South Carolina (SC) Piedmont and SC Coastal Plain sites and locations of the eight Principal aquifers of the United States. Reprinted from Chapelle and others (2016), with permission.

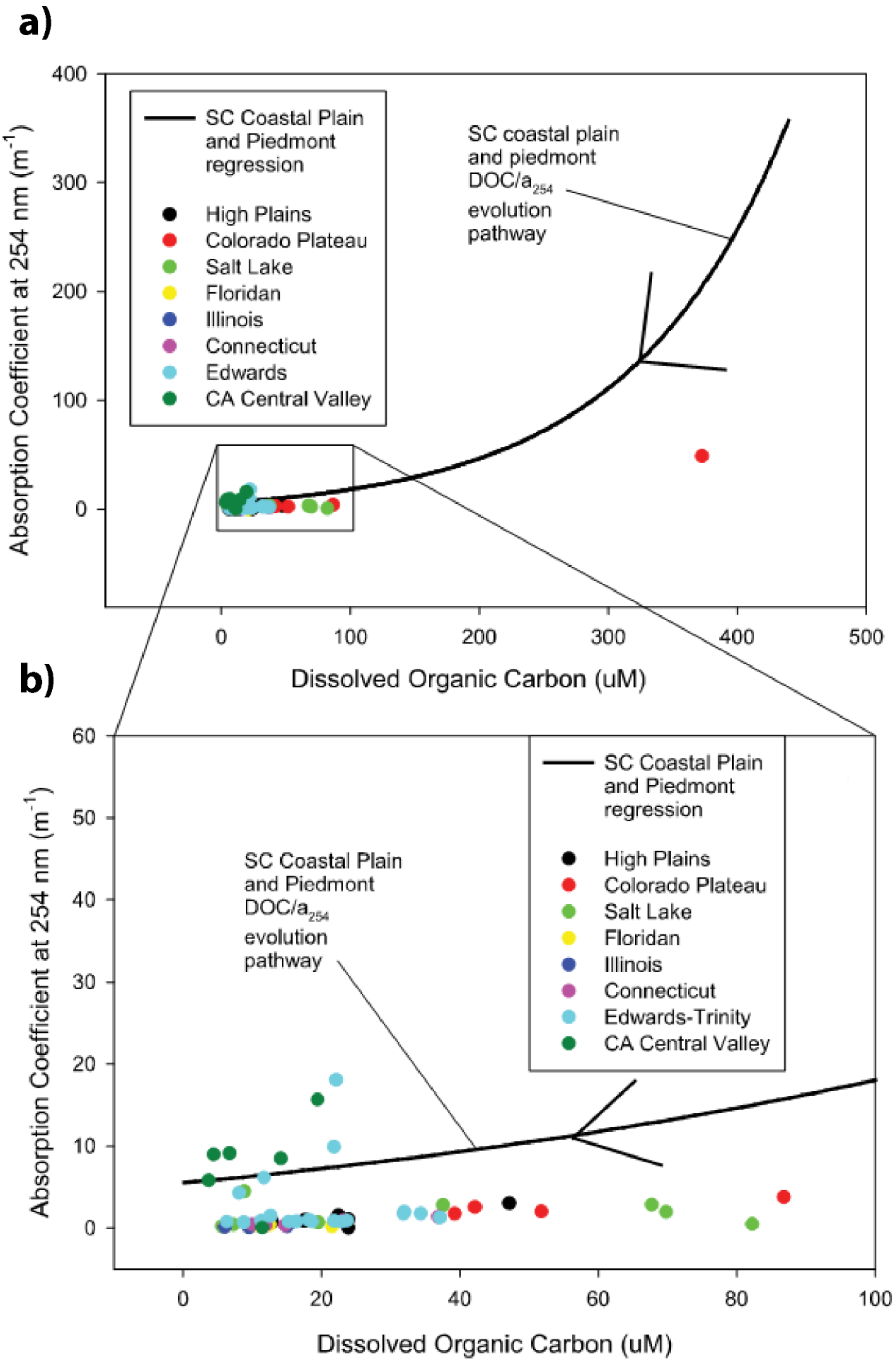


**Figure 16 -** DOC concentrations and UV absorbance coefficients for eight aquifer systems of the United States plotted against: a) the DOC/$a_{\gamma\,254}$ evolution pathway exhibited by the SC piedmont and coastal plain aquifers for the 1-450 $\mu$M ($\mu$mol/L) concentration range; and, b) for the 1-100 $\mu$M concentration range. Reproduced from Chapelle and others (2016), with permission.

Figure 16 illustrates several important characteristics of UV absorbance in groundwater. First, the South Carolina samples were relatively young groundwaters with residence times of ten years or less. In contrast, most of the groundwaters from the other seven aquifers exhibited residence times of greater than 50 years and the absorbance coefficients plot well below the South Carolina DOC/$a_{\gamma\,254}$ pathway. That is consistent with the expectation that biodegradation and sorption processes systematically remove cDOC from groundwater systems. Figure 16 also illustrates another feature of UV absorbance that must be kept in mind when it is applied to groundwater. While most of the samples from the seven aquifers plot below the South Carolina DOC/$a_{\gamma\,254}$ pathway, a cluster of samples from the California Central Valley and the Edwards/Trinity aquifer in Texas plot on or above the South Carolina curve. Those samples are characterized by nitrate concentrations that exceed 10 mg/L, and nitrate, like cDOC, absorbs UV radiation. Dissolved ferrous iron also absorbs UV radiation (Weishaar et al., 2003). For those and other reasons (aromatic content of DOC), UV absorbance is often not a useful surrogate for DOC concentrations in groundwater systems.

One important use of UV absorption measurements in groundwater studies is that they provide an indication of the aromatic composition of cDOC. Weishaar and others (2003) have shown, using a combination of UV absorption measurements and solid state $^{13}$C-NMR measurements, that the aromaticity of DOC is directly proportional to its specific ultraviolet absorbance ($SUVA$), as defined by Equation 2.

$$SUVA_{254} = A_{254}/[DOC] \tag{2}$$

where:

$SUVA_{254}$ = specific ultraviolet absorbance at 254 nm, in units of Liter/mg-m ($L^2M^{-1}$)

$A_{254}$ = absorbance at 254 nm in units of inverse meters ($L^{-1}$)

$[DOC]$ = DOC concentration in units of milligrams per liter ($ML^{-3}$)

Because the bioavailability of DOC decreases as its aromatic composition increases, $SUVA_{254}$ may provide an indication of DOC bioavailability.

## 4.6 Fluorescence Spectroscopy

Fluorescence spectroscopy (also known as fluorimetry or spectrofluorometry) is a type of electromagnetic spectroscopy that analyzes fluorescence from a DOC sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of organic compounds and causes them to emit light at a different wavelength; typically, but not necessarily, visible light. At low DOC concentrations typical of groundwater, the fluorescence intensity will generally be proportional to the concentration of the fluorophore, that is, the part of the DOC that fluoresces. Unlike in UV/visible spectroscopy, ‘standard’ device-independent spectra are not easily attained. Several factors influence and distort the spectra and corrections are necessary to attain 'true', i.e., machine-independent, spectra. This complicates the use of fluorescence spectroscopy for application to groundwater.

An example of how fluorescence spectroscopy has been applied to two hydrologically different aquifers in the United Kingdom was given by Lapworth and others (2008). A Varian$^{TM}$ Cary Eclipse fluorescence spectrometer was used for the fluorescence analysis. Excitation (Ex) wavelengths were set between 200 and 400 nm with a 5 nm bandwidth and emission (Em) wavelengths were set between 250 and 500 nm with a 2 nm bandwidth. The results were entered into what is known as an excitation-emission matrix (EEM) with the excitation wavelength (nm) entered on the x-axis, the emission wavelength (nm) on the y-axis, and fluorescence intensity (au) on the z-axis. Total fluorescence is determined by summing the intensity across the whole EEM after masking interfering peaks such as those for water (Lapworth et al., 2008).

Two study sites tapping Permo-Triassic Sandstones were chosen for this study, one being the Penrith Sandstone of Cumbria and one in the Sherwood Sandstone of South Yorkshire. Both aquifers are regionally important sources of public water supply but have contrasting hydrogeological settings. The Penrith Sandstone is unconfined and is locally recharged directly by atmospheric precipitation. In contrast, the Sherwood Sandstone consists of multiple confined and unconfined aquifers that are more removed from direct atmospheric recharge. Both study sites have oxidizing conditions based on dissolved oxygen concentrations, and therefore changes in fluorescence are not expected to be due to changing redox gradients. Clear differences were observed in the fluorescence profiles of the two aquifers (Figure 17).

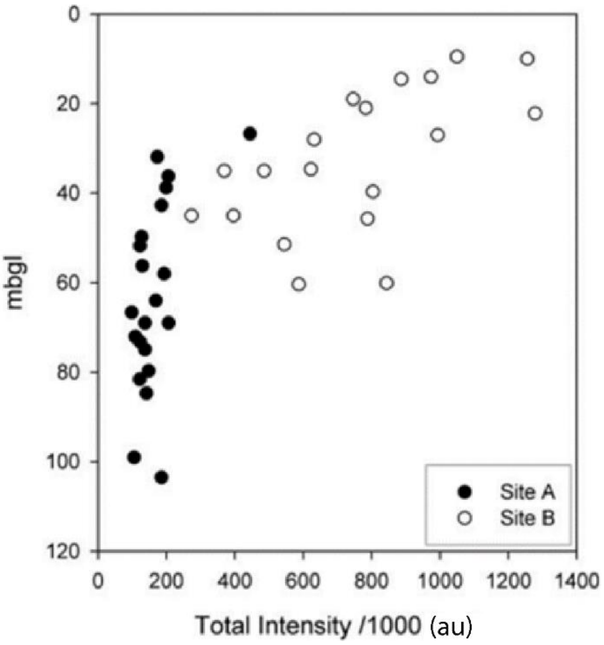


**Figure 17 -** Changes in total fluorescence with depth in the Penrith Sandstone (site A) and the Sherwood Sandstone (site B). Depth is expressed as meters below ground level (mbgl).

First, the total intensity of Penrith DOC was much lower than Sherwood DOC. Secondly, the total intensity of Sherwood DOC decreased initially with well depth and then remained relatively constant. In contrast, the total intensity of Sherwood DOC decreased more gradually with well depth. What could explain these observed differences? One possibility is land use. The Penrith Sandstone is located in a rural setting whereas the Sherwood sandstone is located in a suburban setting. Another possibility is that there are differences in DOC concentrations, based on the strong correlation that was observed between intensity and DOC concentrations ($r^2$ of 0.58, $p = 0.05$). It is also possible that differences in hydrologic setting (confined versus unconfined) may affect the fluorescent properties of DOC. Finally, differences in the lithology between the different sandstones might explain the differences. So, while fluorescence spectroscopy can reveal spatial and temporal differences in the fluorescent properties of groundwater DOC, the significance and/or causes of those differences are difficult to determine.

# 5 Chemical and Biological Processes Controlling DOC Concentrations

Concentrations of DOC in groundwater systems are affected by numerous chemical processes including adsorption, desorption, biodegradation, biosynthesis by microorganisms, and a variety of interactions with aquifer mineralogy. In general, these processes tend to remove DOC as groundwater flows downgradient away from recharge areas. The fact that measurable DOC concentrations are still present in groundwater that has traveled hundreds of kilometers in the subsurface reflects the many potential sources of autochthonous DOC that were discussed in Section 3.2, *Autochthonous Sources of DOC*. This section provides an overview of various chemical and biological processes that affect DOC concentrations in groundwater systems.

## 5.1 Sorption and Desorption

Sorption is the result of a number of processes by which one substance becomes chemically or physically attached to another. In the case of DOC in groundwater, aquifer solids of varying compositions (e.g., quartz sand, limestone, granites) form the solid material to which DOC molecules in aqueous solution can attach. There are many possible kinds of sorption processes for DOC, but they fall into three loosely defined categories of physical, chemical, and electrostatic. Physical sorption processes involve dipole attraction between sorbate and sorbent molecules. The relatively weak bonds associated with physical sorption are often amplified in the case of hydrophobic molecules by their tendency to leave the aqueous phase. Chemical interactions involve covalent bonds and hydrogen bonds that attach DOC to aquifer solids. Finally, electrostatic interactions involve ion-ion and ion–dipole forces that attach DOC to aquifer solids. In the case of DOC, sorption typically reflects the simultaneous contribution of two of more of these mechanisms because the nonpolar or polar character of DOC can vary depending on the source material and the degree of biodegradation to which it has been subjected.

## 5.2 Biodegradation of DOC

DOC, produced by either allochthonous or autochthonous sources, is subject to biodegradation processes immediately upon entering soil or groundwater systems. These biodegradation processes support the extensive and diverse microbial populations found in these environments. In groundwater systems, these microbial populations are predominantly attached to mineral grain surfaces which may indicate that DOC adsorption to those same surfaces facilitates ongoing biodegradation processes. If that is the case, then DOC removal from groundwater by sorption and biodegradation processes are effectively linked.

### 5.2.1 Kinetics of DOC Adsorption and Biodegradation in Groundwater Systems

Groundwater systems have a high capacity for removing DOC from solution. A striking example comes from the Floridan aquifer of southern Georgia, USA (McConnell and Hacke, 1993). The aquifer is being recharged via sinkholes with high-DOC water from the blackwater Withlacoochee River. This river water has a median total organic carbon (TOC) concentration of 1,200 $\mu$mol/L (16 mg/L) and median color of 110 potassium-cobalt units (PCU). Within two hundred meters of the recharge zone, however, TOC concentrations in groundwater decline to less than 300 $\mu$mol/L (3.9 mg/L) and the color decreases proportionally. By the time the "plume" of river-derived water has been transported 10 kilometers downgradient in the aquifer, DOC concentrations have dropped below 80 $\mu$mol/L (1 mg/L) and the color disappears completely (< 1 PCU). This shows that the Floridan aquifer has a substantial capacity to attenuate DOC concentrations, a capacity that reflects both adsorption and biodegradation processes. This implies that the kinetics of DOC removal are relatively rapid.

In some cases, it is possible to quantify the removal kinetics of DOC in groundwater systems due to the combined processes of sorption and biodegradation. An example of how DOC removal kinetics can be quantified was given by Chapelle and others (2016). The aquifer in that study is a crystalline piedmont aquifer in South Carolina, USA that is recharged by water percolating through leave litter on a forest floor. Long-term monitoring showed that DOC concentrations in groundwater increased following rainfall events and decreased in between rainfall events (Figure 18). Those DOC concentration decreases, in turn, provided a way to quantify the DOC removal kinetics.

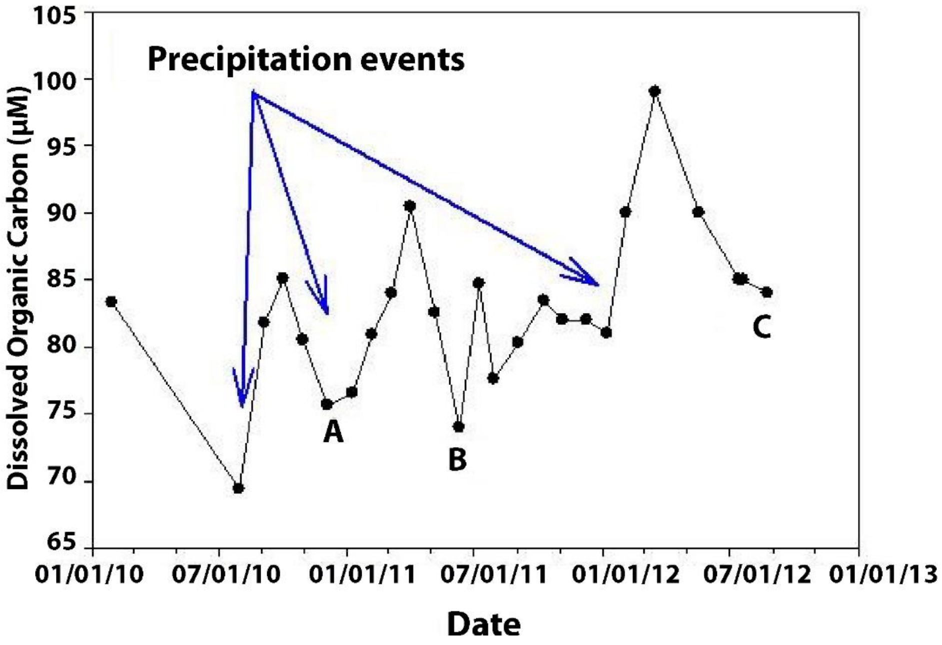


**Figure 18 -** Concentrations of DOC in groundwater from a single well over time in the Piedmont of South Carolina, USA. DOC concentrations increase following precipitation events and then subsequently decline due to sorption and biodegradation processes. Reproduced from Chapelle and others (2016), with permission.

The removal of DOC in between recharge events can be modeled with an equation in which the DOC removal rate constant ($k$) combines the contributions of both biodegradation and sorption as shown on Equation 3.

$$x(t) = C_1 + C_2\, e^{-kt} \tag{3}$$

where:

$x(t)$ = DOC concentrations as a function of time, typically in micromoles per liter ($M/L^3$)

$C_1$, $C_2$ = constants of integration ($M/L^3$)

$k$ = first-order removal rate constant ($T^{-1}$)

$t$ = time since recharge event (T)

When $t = 0$, $x = C_1 + C_2$, which is the initial concentration of DOC in the wellbore following a recharge event. As $t$ becomes larger following the recharge event, the second term of Equation 3 approaches zero and $x(t) \rightarrow C_1$. Therefore, $C_1$ represents DOC that is recalcitrant to biodegradation and sorption and remains in solution after the reactive DOC fraction ($C_2$) has been removed. The estimates of DOC removal in this study ranged from 0.093 to 0.21 micromoles per liter per day ($\mu$mol/L/d) and the DOC removal rates for time periods A, B, and C (Figure 18) ranged from 0.21 to 1.1 percent per day. Assuming a DOC removal rate of one percent per day, then virtually all non-recalcitrant DOC ($C_2$) will be removed from groundwater in less than two years. DOC removal rates of that magnitude, in turn, can fully explain the observed DOC removal in the Floridan aquifer described by McConnell and Hacke (1993). Assuming that similar DOC removal rates are observed in other groundwater systems, these kinetic values provide an explanation for the fact that DOC concentrations in groundwater are typically so low (Leenheer et al., 1974).

# 6 Bioavailability of DOC in Groundwater Systems

Microbially mediated reduction/oxidation (redox) processes, many of which are driven by bioavailable organic carbon, affect the geochemistry and chemical quality of groundwater in both pristine and human-impacted aquifer systems. Methods for determining the bioavailability of particulate and adsorbed organic matter, which are by far the most abundant forms of carbon in groundwater systems, are analytically challenging to perform and difficult to apply in routine hydrologic investigations (Rectanus et al., 2007, Thomas et al., 2012, Chapelle et al., 2012b, Alicea, 2017). In contrast, methods for determining the bioavailability of dissolved organic carbon (DOC) are more straightforward and have been widely applied to groundwater systems (Cronan and Aiken, 1985, Qualls and Haines, 1992, Shen et al., 2015). Importantly, these studies have shown that the behavior of DOC in groundwater is not static, but it interacts continuously and somewhat reversibly with adsorbing aquifer materials such as particulate organic carbon (POC) and mineral surfaces (Davis, 1982; Jardine et al, 1989; Findlay et al., 1993; Hornberger et al., 1994; Findlay and Sobczak, 1996). That being the case, it is possible that the bioavailability of DOC may reflect the bioavailability of the associated POC and AOC compartments at any point in a groundwater flow system.

## 6.1 The Age of Groundwater, Aquifer Sediments and DOC Bioavailability

The model of organic carbon compartment interactions shown in Figure 8 suggests that DOC bioavailability will reflect both groundwater age (time since recharge) and the age of POC and AOC with which groundwater interacts. This prediction can be examined by comparing DOC bioavailability between different aquifers exhibiting substantially different groundwater and sediment POC ages. Two aquifers that meet these criteria are a shallow water table aquifer in the coastal plain of South Carolina (hereafter referred to as SC) and the Central Valley aquifer system of California (hereafter referred to as CA).

Groundwater from SC receives recharge directly through modern agricultural soils with relatively high amounts of organic carbon (1 to 5 weight percent; 10,000 to 50,000 mg/kg), the water table varies from 1 to 3 m below land surface, and the groundwater ranges in age (time since recharge) from one to five years (Puckett and Hughes, 2005). The SC aquifer sediments are Holocene (~10,000 BP) in age. In contrast, groundwater from the CA site is much deeper (100 to 300 m), contains lower amounts of POC (~0.1-0.5 weight percent; ~1,000 to 5,000 mg/kg) in sediments of Pliocene to Pleistocene age (2 to 5 million years), and the groundwater age ranges from several hundred to several thousand years (Landon and Belitz, 2008).

The bioavailability of DOC from these two aquifer systems was compared using eight different indicator parameters (Chapelle et al., 2012b). These eight indicators are:

1) concentrations of total hydrolyzable neutral sugars (THNS) of DOC;
2) mole percent glucose (a sugar) of THNS;
3) concentrations of total hydrolyzable amino acids (THAA) of DOC;
4) mole percent glycine (an amino acid) of THAA;
5) initial bacterial counts;
6) bacterial growth over time during incubation;
7) specific ultraviolet absorbance at wavelength 254 nM ($SUVA_{254}$); and,
8) bioassays of carbon dioxide production/consumption over time during incubation.

Concentrations of total hydrolyzable neutral sugars (THNS) present in DOC have been shown to be proportional to DOC bioavailability (Volk et al., 1997; Weiss and Simon, 1999; Routh et al., 2001; Benner, 2003) and may be an indicator of bioavailability (Indicator 1). It has also been observed that the mole percent glucose of THNS increases between young surface ocean waters and ancient deep ocean waters (Benner, 2003). A higher mole percent glucose, therefore, may indicate lower DOC bioavailability (Indicator 2). Concentrations of total hydrolyzable amino acids (THAA) present in DOC are positively correlated with bioavailability (Dauwe et al., 1999, Benner, 2003), so that higher THAA of DOC may indicate higher bioavailability (Indicator 3). The amino acid glycine has been observed to become enriched in DOC as biodegradation proceeds (Dauwe et al., 1999), so that a higher mole percent glycine of THAA may be associated with lower bioavailability (Indicator 4). Numbers of bacterial cells present in water (Indicator 5) have been used as a qualitative indicator of available carbon and nutrients in groundwater (Marxsen, 1988). Similarly, bacterial growth rates during incubation (Indicator 6) have commonly been used as an indicator of DOC bioavailability in both groundwater (Hirsch and Rades-Rohkohl, 1988) and surface-water systems (Kroer, 1993). Naturally occurring DOC is a complex mixture of aromatic and aliphatic organic compounds (Aiken, 1989). It has been observed that as DOC is subjected to biodegradation, the aliphatic portion tends to be preferentially utilized relative to the aromatic portion (Sun et al., 1997). This, in turn, increases the aromaticity of the remaining DOC which reflects decreased bioavailability. It has been shown (Weishaar et al., 2003) that the aromaticity of DOC is proportional to the specific ultraviolet absorbance at wavelength 254 nanometers ($SUVA_{254}$). Thus, higher values of $SUVA_{254}$ imply higher DOC aromaticity and thus lower bioavailability (Indicator 7). Finally, microbial metabolism of DOC can result in either the production or consumption of carbon dioxide. Heterotrophic bacteria growing or maintaining biomass can result in the net release of carbon dioxide during incubation (McDowell et al., 2006). Alternatively, heterotrophic bacteria also have the capability to fix carbon dioxide in order to build biomass (Šantrůčková et al., 2005) which can lead to a net consumption of carbon dioxide. Both carbon dioxide production (McDowell et al., 2006) and consumption (Roslev et al., 2004) during incubation have been used as indicators for the bioavailability of DOC in surface-water systems. Thus, it is the net *change* of carbon dioxide during incubation that is a potential indicator of DOC bioavailability (Indicator 8).

Table 1 shows the observed differences between the populations for each indicator parameter (greater or less than) and whether those differences are consistent with the hypothesis that SC DOC is more bioavailable than CA DOC. Also shown in Table 1 is the statistical significance of the differences between the CA and SC sample populations. All of the indicators are consistent with the supposition that the SC samples were more bioavailable than the CA samples, although the statistical significance of mole percent glucose of THNS, mole percent glycine of THAA, and final cell count is not conclusive.

**Table 1 -** Comparison of bioavailability indicators measured in groundwater produced from wells in California and South Carolina.

| **Bioavailability Indicator** | **California Median, $Q_{25}$, $Q_{75}$** | | | **South Carolina Median, $Q_{25}$, $Q_{75}$** | | | **Differences**[1] | **Statistical Significance**[2] |
|---|---|---|---|---|---|---|---|---|
| THNS (nmol/L) | 70.8 | 66.6 | 73.4 | 196.7 | 93.6 | 268.4 | yes | 0.108 |
| Mole percent glucose of THNS | 66.8 | 60.1 | 70.2 | 28.7 | 21.9 | 56.8 | yes | 0.059 |
| THAA (nmol/L) | 38.6 | 25.7 | 74.0 | 246.6 | 125.4 | 331.2 | yes | 0.008 |
| Mole percent glycine of THAA | 48.2 | 33.7 | 70.5 | 30.5 | 24.4 | 43.0 | yes | 0.228 |
| Initial cell count (cells/ml x $10^4$) | 0.62 | 0.53 | 0.85 | 1.5 | 1.2 | 1.7 | yes | 0.003 |
| Final cell count (cells/ml x $10^4$) | 1.1 | 0.8 | 6.6 | 7.1 | 1.9 | 11.7 | yes | 0.228 |
| SUVA 254 | 4.0 | 2.3 | 6.2 | 0.95 | 0.59 | 1.27 | yes | 0.001 |
| $CO_2$ Change during incubation (mg/L) | 3.3 | 1.4 | 7.2 | 13.3 | 10 | 24.6 | yes | 0.006 |

[1] Differences consistent with SC DOC being more bioavailable than CA DOC?
[2] Statistical significance of the difference between populations (p value).

## 6.2 The Age of Aquifer Sediments and DOC Bioavailability

The model of organic carbon compartment interactions shown in Figure 8 of Section 2.2 also suggests that the bioavailability of DOC should reflect the age of POC and AOC sources to an aquifer and not just groundwater age. One would expect, therefore, that DOC present in groundwater presently receiving recharge through active modern soil zones will be more bioavailable than groundwater of the same age receiving recharge from much older sediments. Two shallow water table aquifers exhibiting these differences are located at Naval Submarine Base Kings Bay (hereafter referred to as KB) in the coastal plain of Georgia and the Naval Air Warfare Center in New Jersey.

The KB site is a sandy aquifer of marginal marine origin receiving modern atmospheric recharge that passes through an organic-rich backwater swamp deposit of late

Miocene age (5 to 6 million years BP) that is two to three meters thick. The NAWC site, in contrast, is a bedrock aquifer of Triassic age that receives modern atmospheric recharge passing through an organic-rich modern soil zone that is also two to three meters thick. Groundwater at both sites is anoxic (dissolved oxygen < 0.05 mg/L) due to these POC and AOC sources. That simply indicates that the amount of bioavailable carbon exceeds the flux of oxygen being delivered to each aquifer. It does not, however, indicate any differences in DOC bioavailability between the two sites.

The bioavailability of DOC in groundwater from these two aquifers was compared using biochemical indicators similar to those discussed in Section 6.1 but including the ratio of DOC to total nitrogen (DOC:TN ratio). The DOC:TN ratio has been widely studied in the context of leaf litter biodegradation in forested soils because leaf litter decomposition drives nutrient cycling in forested ecosystems. Surprisingly, it has been consistently found that microbial respiration rates, as measured by $CO_2$ production, *increase* with increasing C:N ratios (Ohtonen, 1994; Gödde et al., 1996; Michel and Matzner 2002; review by Spohn, 2015). That result is counterintuitive, as it might be expected that increasing amounts nitrogen would have a “fertilizer” affect, thus increasing microbial respiration rates. Gödde and others (1996) hypothesized that less bioavailable DOC would be expected to contain a higher proportion of lignin (a component of plant cells). The low C:N ratios characteristic of lignin would then require soil microorganisms to cycle more C in order to obtain their N requirements, thus increasing $CO_2$ production. Whatever the underlying mechanism(s) are, however, the much higher DOC:TN ratios in groundwater from the NAWC site than the KB site (Figure 19, Figure 11) indicates greater DOC bioavailability. Furthermore, because DOC and nitrogen species are routinely measured in studies of groundwater chemistry, it may be that the DOC:TN molar ratio can be a useful indicator for comparing DOC bioavailability between different aquifer systems.

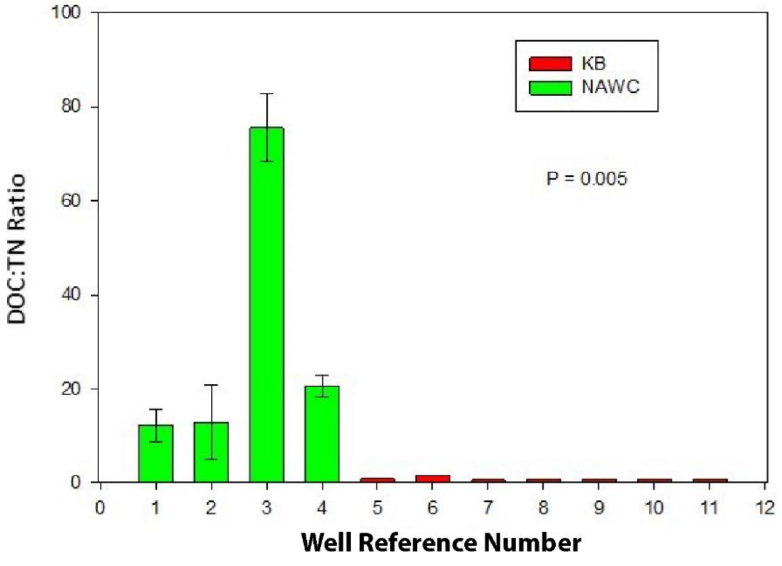


**Figure 19 -** DOC:TN molar ratios in groundwater suggesting greater DOC bioavailability at NAWC relative to the KB site.

Consistent with the apparent differences in DOC bioavailability inferred from biochemical indicators discussed above, a strong correlation between DOC and dissolved inorganic carbon (DIC), a product of DOC mineralization, was observed in groundwater from the NAWC site (Table 2). In contrast, no significant DOC-DIC correlation was observed at the KB site. Because DIC is produced directly by oxidation of bioavailable DOC, the presence or absence of such a correlation is a potential indicator of differences in DOC bioavailability between different aquifers. The DOC/DIC correlation for both the NAWC and KB sites, as expressed by the coefficient of determination ($r^2$), is shown on Table 2 along with the other biochemical indicators.

Importantly, the DOC:TN ratio and the correlation of DIC with DOC (Table 2) are bioavailable indicators based solely on water-chemistry measurements routinely made in groundwater quality studies and do not require specialized laboratory facilities. As such, they may be the most accessible DOC bioavailability indicators available to field hydrogeologists.

**Table 2 -** Comparison of bioavailability indicators between the Kings Bay and NAWC sites.

| Bioavailability indicator | Mean Value Kings Bay | Mean Value NAWC | Consistent?[1] | Statistical significance[2] |
|---|---|---|---|---|
| THNS yield | 0.55 | 1.14 | yes | 0.003 |
| Mole percent of THNS | 18.15 | 31.50 | yes | 0.001 |
| THAA yield | 0.42 | 1.32 | yes | 0.004 |
| Mole percent glycine of THAA | 38.13 | 29.37 | yes | 0.019 |
| C/TN ratio | 0.81 | 23.19 | yes | 0.003 |
| DOC-DIC correlation ($r^2$) | 0.72 | 0.18 | yes | 0.003 |

[1] Consistent with NAWC DOC more bioavailable than KB DOC?
[2] Statistical significance of difference (p value).

# 7 Concluding Remarks

## 7.1 Summary

Concentrations of DOC have been measured reliably in groundwater since the early 1970s. Since then, these measurements have been made for a wide variety of aquifer systems for a variety of scientific purposes. Several investigators working in arid environments in the western United States, for example, discovered that DOC transported by shallow groundwater to seasonal streams was an important contributor to the structure of local ecosystems (Hornberger et al., 1994; Findlay and Sobczak, 1996; Boyer et al., 1997; Baker et al., 2000). Other investigators viewed DOC as an important contributor to observed changes in groundwater chemistry as it flowed downgradient in regional aquifers (McMahon and Chapelle, 1991a), and how it contributes to aquifer diagenesis (McMahon et al., 1992). When the bioremediation of anthropogenic contaminants became an important environmental issue in the 1990s, DOC began to be considered as a competing substrate, negatively affecting the biodegradation of petroleum hydrocarbons but positively affecting the biodegradation of chlorinated solvents (Wiedemeier et al., 1999).

Because of this variety of issues and approaches, a consensus concerning the overall hydrologic, ecologic, and geochemical significance of DOC in groundwater has been slow to emerge. Beginning with studies of DOC dynamics in soils and groundwater (Cronan and Aiken, 1985; Jardine et al., 1989), it gradually became clear that DOC was adsorbing rapidly and reversibly with soil components such as ferric hydroxides and POC. This provided an explanation for DOC's observed behavior in groundwater, including its rapid removal and remobilization depending on hydrologic conditions. In addition, these adsorption processes explained the ubiquitous low concentrations of DOC in groundwater not directly impacted by surface water sources that had been observed early on (Leenheer et al., 1974).

All of this led to the view, first expressed by Aiken (1989), that groundwater systems behave like naturally occurring chromatographic columns in which components of DOC are systematically separated depending on their chemical and physical properties (Shen et al., 2015; Figure 8). Like chromatographic columns, these adsorption processes are partially reversible and there is a continuous interplay between the adsorbed and dissolved compartments. Unlike chromatographic columns, however, a variety of microbially-mediated redox processes are superimposed on these sorption-desorption processes. Those redox processes oxidize DOC, POC, and AOC with the sequential reduction of dissolved oxygen, ferric iron, and sulfate, and carbon dioxide. The net result of these redox processes is to increase concentrations of dissolved solids in groundwater as it flows along the hydrologic gradient while simultaneously decreasing the bioavailability of the remaining DOC, POC., and AOC.

The literature reviewed in this book suggests that the composition and bioavailability of DOC in groundwater reflect the bioavailability of the associated POC and

AOC compartments as well. The chief hydrologic and geochemical significance of DOC may be that it indicates the bioavailability, and thus the reducing capacity, of total organic carbon present in aquifer systems. That reducing capacity, which determines the kinds of reduction/oxidation processes that can occur, is a principal driver for the geochemical processes that determine groundwater quality in both pristine and human-impacted aquifer systems.

## 7.2 Groundwater Under the Direct Influence of Surface Water (GWUDISW)

It is widely recognized that wells producing water with a substantial component of surface water from streams, rivers or lakes present risk of harm to users who drink that water. Surface water often contains harmful biological constituents such as pathogenic bacteria including parasites and viruses. Thus, it is generally accepted that when surface water is used for drinking water it should be treated to render the water safe. In contrast, groundwater pumped from wells is generally considered to be safe for drinking unless there are extenuating circumstances associated with the wells. One such circumstance is the presence of water that comes from surface water if the potentially harmful constituents are not sufficiently attenuated as the water travels from the surface water body to the well.

The United States Environmental Protection Agency (USEPA) established a well category designated as GWUDISW in 1992 and many states in the USA, provinces in Canada and elsewhere have issued guidance documents or regulations for this category of well. These documents provide advice on how to recognize such wells and, when recognized, provide regulations concerning water treatment to make the well water safe for drinking. However, these guidelines for identification of GWUDISW wells do not include mention of any role for DOC analyses. Given the following:

- groundwater pumped from wells used for drinking water with no indications of contamination (e.g., pristine) consistently has DOC in the range of 0.3-0.7 mg/L;
- DOC in such wells is rarely above 5 mg/L;
- surface waters typically show thousands of mg/L DOC; and,
- the cost of DOC analysis is low;

it would be appropriate to include DOC as one of the criteria for identification of GUIDISW wells. The presence of anomalously high DOC is not proof of a risk from surface water but, is a sufficiently strong indicator to justify more investigation. DOC above 1 mg/L is reason for concern. DOC above 5 mg/L is reason to investigate further to determine whether other evidence indicates a GWUDISW which would require treatment if used for drinking water.

Figure 20 shows a pumping well that draws water both from recharge through the vadose zone and from a river. Whether or not the well water will have a high enough concentration of DOC to indicate a substantial contribution of river water depends on the level of DOC in the river water, the degree of degradation of the DOC in the water as it flows to the well and the relative portions of river/non-river water entering the well.

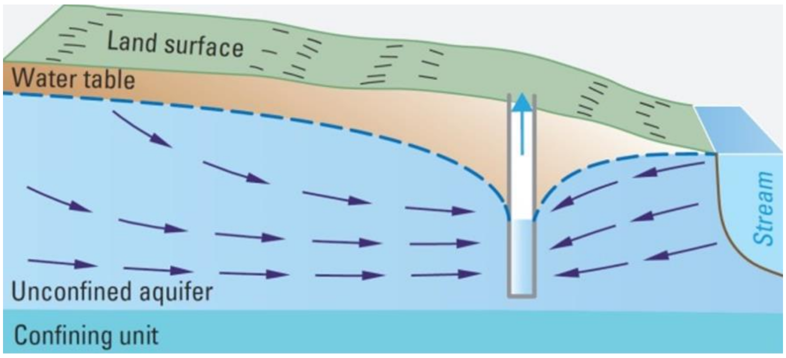


**Figure 20 –** Cross-sectional view of a pumping well near a river illustrating that water in the well is a mix of water from recharge that infiltrates through the vadose zone and water that seeps from the river into the aquifer (from Konikow and Bredehoeft, 2020).

## 7.3 Topics for Further Study

The chief hydrologic and geochemical significance of DOC may be that it indicates the bioavailability, and thus the reducing capacity of total organic carbon present in aquifer systems. That reducing capacity is a principal factor determining the groundwater quality of both pristine and human-impacted aquifers. Continuing study of interactions between all forms of organic carbon (DOC, POC, AOC), therefore, may improve assessments of groundwater quality in the future.

# 8 Exercises

## Exercise 1

Convert the units of DOC concentration in milligrams per liter (mg/L) to micromoles per liter ($\mu$mol/L).

Click for solution to Exercise 1⤵

## Exercise 2

What DOC concentrations are typical of groundwater systems that are not under the direct influence of surface water?

Click for solution to Exercise 2⤵

## Exercise 3

What were the four principal conclusions made in the first systematic study of DOC concentrations in different aquifers throughout the United States (Leenheer et al., 1974).

Click for solution to Exercise 3⤵

## Exercise 4

What are the three principal sources of DOC to groundwater?

Click for solution to Exercise 4⤵

## Exercise 5

What two analytical methods for characterizing DOC are based on the optical properties of organic carbon?

Click for solution to Exercise 5⤵

## Exercise 6

Surface and soil waters recharging aquifers typically have relatively high DOC concentrations (10 to 20 mg/L; ~830 to 1,660 µmol/L). At what rate is DOC in aquifer recharge water typically removed from groundwater in units of percent per day?

Click for solution to Exercise 6⤵

## Exercise 7

What is the most commonly used method for assessing the bioavailability of DOC in groundwater?

Click for solution to Exercise 7⤵

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# 10 Exercise Solutions

## Solution Exercise 1

One milligram per liter of DOC divided by the atomic weight of carbon (12) times 1,000.

$$\frac{1\frac{\mathrm{mg}}{\mathrm{L}}}{12\frac{\mathrm{g}}{\mathrm{mole}}1{,}000\frac{\mathrm{mg}}{\mathrm{g}}}\,1{,}000{,}000\,\frac{\mu mol}{mol} = 83.3\frac{\mu mol}{L}$$

**Return to Exercise 1**↥

## Solution Exercise 2

Typically, less than 1 mg/L (83.3 $\mu$mol/L).

**Return to Exercise 2**↥

## Solution Exercise 3

Based on that study, Leenheer and others (1974) made four principal observations:

1) DOC concentrations in groundwater were generally much lower than commonly found in surface waters, with median concentrations ranging between 0.5 and 0.7 mg/L (41.7 to 58.3 $\mu$mol/L);
2) a shallow aquifer in Florida receiving active recharge from surface-water sources had much higher DOC concentrations (15 mg/L; 1,250 $\mu$mol/L) than wells tapping the deeper Floridan aquifer (1.4 to 0.1 mg/L; 117 to 8.3 $\mu$mol/L) that was not immediately influenced by surface water;
3) there were no statistically significant differences in DOC concentrations between aquifers of differing lithologies; and,
4) there were clear statistical correlations between DOC concentrations, specific conductance, and alkalinity.

**Return to Exercise 3**↥

## Solution Exercise 4

There are three principal sources of DOC to groundwater. The first are allochthonous sources derived from plant material at land surface and transported to aquifers by infiltrating recharge water. Allochthonous DOC is typically the major source of DOC to shallow water table aquifers. The second major source is autochthonous DOC derived from particulate organic carbon that was buried with aquifer sediments at the time of deposition. Autochthonous DOC is typically a more important source of DOC to deeper confined aquifers. Finally, there are anthropogenic sources (carbon derived from human activities) of DOC as well. These anthropogenic sources include chemical contaminants such as human sewage, petroleum hydrocarbons or chlorinated solvents, DOC derived from agricultural activities, and animal husbandry practices.

Return to Exercise 4↥

## Solution Exercise 5

UV absorption and UV fluorescence.

Return to Exercise 5↥

## Solution Exercise 6

Between 0.2 and 1 percent per day.

Return to Exercise 6↥

## Solution Exercise 7

Bioassays of carbon dioxide production/consumption over time during incubation of groundwater samples.

Return to Exercise 7↥

# 11 Notations

| | |
|---|---|
| $A_{\gamma}$ | measured UV absorbance at 254 nm ($L^{-1}$) |
| $A_{254}$ | absorbance at 254 nm in units of inverse meters ($L^{-1}$) |
| $\alpha_{\gamma}$ | absorption coefficient ($L^{-1}$) |
| $C_1$, $C_2$ | constants of integration ($M/L^3$) |
| $[DOC]$ | DOC concentration in units of milligrams per liter ($ML^{-3}$) |
| $k$ | first-order removal rate constant ($T^{-1}$) |
| $r$ | Path length (L) |
| $SUVA_{254}$ | specific ultraviolet absorbance at 254 nm ($L^2M^{-1}$) |
| $t$ | time since recharge event (T) |
| $x(t)$ | DOC concentrations as a function of time, typically in micromoles per liter ($M/L^3$) |

# 12 About the Author

**Dr. Francis H. Chapelle** was educated at the University of Maryland (Bachelor of Arts in Music and Bachelor of Science in Geology) and the George Washington University (Master of Science and Doctor of Philosophy in Geology). He was a Research Hydrologist with the United States Geological Survey from 1979 to 2021. His research interests focus on how microbial processes affect the chemistry of groundwater in both pristine and contaminated aquifer systems. He is the author of the textbook *Ground-Water Microbiology and Geochemistry, 2nd Edition* (John Wiley & Sons, 2003), and author of a book for the general reader *The Hidden Sea* (The National Groundwater Association, 2000) describing groundwater and groundwater systems. His research received a National Award for Environmental Sustainability given by Renew America, Washington D.C. (1996), the O.E. Meinzer Award in Hydrogeology given by the Geological Society of America (2000), and the Distinguished Service Award given by the U.S. Geological Survey (2008).

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# Modifications to Original Release

## Changes from the Original Version to Version 2

Original Version: August 2022, Version 2: December 2022

### General changes:

Page breaks were corrected in the front material

Modification section was added

Table of Contents was updated after revision

### Specific changes:

Page numbers refer to page numbers in the original pdf.

page 18 and 19, references to Figure 3 were corrected to be references to Figure 11

page 22, the caption of figure 13 was removed from the content of the paragraph that references Figure 13.

page 26, 3rd line from bottom, Figure 6 was corrected to Figure 14

page 39, missing words were inserted between 'Table 1' and 'is' such that it now reads: 'Table 1 shows the observed differences between the populations for each indicator parameter (greater or less than) and whether those differences are consistent with the hypothesis that SC DOC is more bioavailable than CA DOC. Also shown in Table 1 is'

## Changes from Version 2 to Version 3

Version 3: January 2023

### General changes:

Throughout book, each $\mu$mole changed to $\mu$mol, and nmole changed to nmol

### Specific changes:

Page numbers refer to the pdf of Version 2

page i, added s to http of the first two gw-project.org links

page iii, hyperlink added to doi

page vii, indented first line of last paragraph

page 1, end of caption, Figure 1, changed '– DOC –' to (DOC)

page 16, 1st paragraph, 7th line, first μ changed to italics to be consistent with the rest of the book

page 28, in Equation 1 and associated variable definitions, the subscript of a and A was changed from $\Upsilon$ to $\gamma$ match the text

page 30, caption of Figure 16, 2nd line, the a of $a_{\gamma\,254}$ was changed to italic font

page 31, parameters definitions for Equation 2, units and dimensions for $SUVA_{254}$ corrected from dimensionless to Liter/mg-m ($L^2M^{-1}$)

page 31, brackets added to *DOC i.e.,* [*DOC*] in parameter list to indicate concentration

page 33, line 8, variables r and p changed to italic $r$ and $p$

page 36, last paragraph, line 8, per cent changed to percent

page 37, 3rd paragraph, line 7, corrected unmatching parentheses, '(~0.1 0.5 weight percent (~1,000 to 5,000 mg/kg)' changed to '(~0.1 0.5 weight percent; ~1,000 to 5,000 mg/kg)'

page 41, last row of Table 2, variable r changed to italic

page 54, Solution Exercise 1, removed italics from equation units

page 56, corrected dimensions for $A_r$ to ($L^{-1}$) and $SUVA_{253}$ to ($L^2M^{-1}$)

page 56, corrected *DOC* to [*DOC*]

## Changes from Version 3 to Version 4

Version 3: January 29, 2023, Version 4: January 25, 2024

Page numbers refer to the pdf of Version 3

page ii, added page requesting support of the Groundwater Project

page ii, now page iii, updated version number and date

page iii, now page iv, added "Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government."

page xi, corrected spelling of Blotevogel

page 32, added a page break before Figure 17 so the full cation would be on the same page as the figure