Chemical Composition and Bioavailability of Dissolved Organic Nitrogen in Atmospheric Wet Deposition from Urban and Rural New Jersey Sites

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Executive Summary

Atmospheric deposition is a major source of nitrogen to many ecosystems. Marked changes in both terrestrial and aquatic ecosystems are occurring as a result of increased nitrogen (N) deposition from anthropogenic sources. To date, most studies of the magnitude, sources and effects of atmospherically deposited N have only considered inorganic-N. However, a considerable portion of N in rainwater is in the form of organic-N, and almost nothing is known of the chemical composition, sources or ecosystem effects of the bulk of that organic-N. In addition to the N content of organic matter, polar organic compounds are of interest for additional scientific and societal reasons, including the changing fluxes of carbonaceous matter between the atmosphere and the Earth's surface, impaired atmospheric visibility and poor air quality, influence on cloud properties (and climate) due to interactions with atmospheric water, deposition of acidic compounds to ecosystems, and effects on human health. Thus, is it important to understand the full suite of atmospheric organic compounds (both with and without N).

Dissolved organic matter (DOM) present in the atmosphere is highly complex and poses a significant analytical chemical challenge. No single analytical method has been able to detect and quantify single DOM compounds directly in a rainwater sample, and typically extensive sample manipulation is required before analysis of even single compounds. The research conducted in this project focused on the development and application of new analytical approaches (electrospray ionization mass spectrometry: ESI-MS) to directly measure and characterize the chemical composition of the suite of DOM compounds in atmospheric wet deposition. The chemical structure of compounds is key to identifying the sources (processes and technologies) and effects of atmospheric DOM.

Our long-term goal is to identify the current, and potentially future, processes (natural and anthropogenic) and technologies that are major contributors to atmospherically deposited organic matter and to assess the effect of these specific chemicals on receptor ecosystems. This project was an important step toward that long-term goal.

The specific objectives of the studies conducted were to:

- **characterize the chemical composition of total dissolved N, both inorganic and organic nitrogen, in atmospheric deposition in 1 urban and 1 relatively undisturbed site in New Jersey;**
- **begin to identify sources of organic matter in atmospheric deposition at those sites; and**
- **identify which of the organic compounds, and therefore which potential sources, are bioavailable and thus contributing to ecological changes in ecosystems.**

This report presents the results and interpretation to date for data generated during this 3 year project (NJDEP Contract #SR01-079, SR02-021 and SR03-036).

Approach

Precipitation Collection and Analysis. Rainwater was collected at an urban site (Camden) and at a rural (Pinelands) site in south-central New Jersey. During the first 24-month project period (June 2001 – May 2003) samples were collected during most rain events in all seasons. The total number of rainwater samples collected during that time was 139 (61 Camden and 78 Pinelands) and almost all storms were from a SW or WSW direction. During June 2003 - March 2004 sample collection was targeted for storms from a SE, E, NE, or NW direction; eight samples were obtained. All samples were analyzed for the following bulk chemical properties: pH, ammonium, nitrate plus nitrite, soluble inorganic phosphate, total dissolved organic N (DON) and C (DOC). Over 800 analyses were completed of these bulk chemical properties. Detailed molecular level chemical characterization of the suite of dissolved organic matter (DOM) compounds in the rainwater samples was conducted using a new analytical approach, electrospray ionization mass spectrometry (ESI-MS). Considerable new methods development was involved in the use of ESI-MS for DOM in rainwater.

One hundred (100) rainwater samples were analyzed by ESI-MS (48 Camden; 52 Pinelands). All ESI-MS data were compiled into a database for interpretation. The database contains *over 1 million data points*. In addition, approximately another 1.5 million data points were generated by ESI-MS in this project for standards, blanks, UV oxidized samples, sample spikes, and following HPLC separation. This is the most extensive molecular level database available for rainwater DOM chemical composition. New data processing software routines were developed to compile and analyze this large database; this new software reduces the data processing time by ~80%.

Authentic standards of 80 compounds of atmospheric significance were analyzed by ESI-MS to establish ESI response to compound functional groups and to develop response factors for known compounds. A Molecular Marker Database of Atmospheric Organic Compounds was developed (with entries for over 1000 compounds). This Molecular Marker database, in concert with authentic standards of selected compounds, are keys to interpreting the ESI-MS data and to identifying and quantifying sources of DOM in rainwater.

Bioavailable DOM Compounds. Utilization of DOM by aquatic microbial communities was examined for Camden and Pinelands rainwater. The bioavailable and refractory pool of DOM was characterized, both from bulk chemical analysis as well as ESI-MS molecular level chemical characterization.

Chemical Composition of Rainwater DOM at 2 New Jersey Locations

Bulk Chemical Composition and Patterns. Inorganic N, phosphate, DON, and DOC exhibited a general seasonal trend of higher concentrations in summer and lower concentrations in winter. There were no strong differences in concentration between the Camden and Pinelands sites. pH also exhibited a seasonal pattern, but with lower pH in summer and higher in fall through spring. There appeared to be an overall trend of increasing pH over the project period at both locations. Inorganic N concentrations were similar to those reported for the two NADP sites in New Jersey (Washington Crossing site ID NJ99 and Edwin Forsythe National Wildlife Refuge ID site NJ00) over the same period.

Interpretation of DOM Composition in New Jersey Rainwater. A large number of DOM compounds occur in rainwater at the two New Jersey sites. Overall, 862 unique m/z (compounds at unit mass resolution) were detected by ESI-MS in the 92 precipitation samples collected during the initial 24-month sampling period. This constitutes the C&P ESI-MS rainwater database.

Approximately 500 unique m/z occurred in >20% of the samples from each season and site and were therefore retained for further interpretation.

The DOM compounds are relatively low molecular weight, and ranged from approximately 50 to 620 amu. Most masses detected clustered between 100 and 400 amu.

More of the compounds detected had basic properties than acidic properties. There were more compounds (305) with structures containing –CH3, =O, $-NH_2$, and N heterocycles (basic-type functional groups), than compounds (194) containing –COOH, -NO2 (acidic-type functional groups). Detection of compounds with these functional group types was confirmed by analysis of >80 authentic standards. This analysis provides considerable insight into the chemical structure of each compound and indicates that carboxylic acids and amines do not constitute the majority of compounds.

A range of anthropogenic and biogenic source types for the organic compounds are indicated. Our Molecular Marker Database of Atmospheric Organic Compounds was used to make preliminary matches with the most frequently occurring m/z (compounds) in the rainwater samples. Matches were based on a combination of compound molecular weight, functional group characteristics, and response of authentic standards. These preliminary matches indicated a range of anthropogenic (e.g., automobile exhaust, tobacco smoke, coal combustion, diesel, chemical manufacture) and biogenic (vegetation, biomass burning) sources types.

Groups of compounds were identified which show similar patterns of occurrence with season and with site. These patterns suggest similar source(s) for compounds in a group. The two most obvious patterns were those compounds: 1) with and 2) without seasonal patterns in occurrence. The most dominant pattern identified to date is the group of compounds with no obvious seasonal pattern in occurrence and with generally high ion abundance (i.e., concentration). This group (Category II compounds) included 95 masses. Many other masses showed no seasonal pattern as well, but had lower frequency of occurrence. The lack of a season pattern in occurrence (such as in Category II compounds) suggests that these compounds are not from natural, biogenic sources, but rather from anthropogenic sources. Given the very different land use (highly urban versus forested) at these two sites, the result also suggest that many of these compounds are from sources remote to both sites, and transported to the site from long-range atmospheric transport. A smaller number of compounds showed an obvious seasonal pattern, with higher occurrence in the summer than in the fall and winter (Category III and IV compounds). The seasonal pattern of higher occurrence during growing seasons, with decreasing frequency in non-growing seasons, may be indicative of natural, biogenic sources. These could be local and/or longer-range sources. It is also possible that these compounds are from anthropogenic sources with a season production pattern. Secondary photochemical reactions may also be a factor in the pattern of occurrence of these masses.

Approximately 90% of the samples obtained during the 24-month period were from storms with a southwesterly trajectory. Eight samples (4 each from Camden and Pinelands) were obtained the following year from storms from other trajectories (SE and NW direction). In general, there were numerous similarities in chemical composition between storms from a southwesterly direction and those form the SE or NW. The overall molecular weight distribution of compounds was similar and many of the same masses were detected. There were also notable differences. In particular, numerous masses were not detected in the SE or NW storms that had a high frequency of occurrence in the southwesterly storms. A number of masses had high frequency of occurrence in the SE storms that only occasionally occurred in the southwesterly storms. While a number of caveats must be considered in comparing these two sets of samples, as noted in the report, the results suggest that storms with different trajectories have notable differences in organic matter chemical composition, indicative of some differences in sources.

Additional analytical approaches were explored to obtain further identification of specific compounds in the rainwater samples. This included analyzing samples and standards across a range of ESI-MS instrument operating conditions and coupling liquid chromatographic separation (HPLC) to the ESI-MS analysis. Potential compounds from different source types were chosen for these advanced analyses. The results from this combination of approaches further suggested the occurrence of a range of specific anthropogenic and natural source types contributing to the rainwater organic matter. However, combining these advanced approaches with the ESI-MS proved to be very time consuming and as such identification of only a limited number of masses (m/z's) could be explored. In addition, because of the very low concentrations of any one compound and the increased background noise introduced with the HPLC, the results were often difficult to interpret with certainty.

We advise that the next step in obtaining positive identification of the large suite of specific organic compounds in rainwater, and therefore to further the identification of source-types, is to continue to use ESI-MS, but to move to the use of ultrahigh resolution ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR MS). The suggested use of FT-ICR is based on work conducted outside of the present NJDEP project in which we analyzed DOM from a number of aquatic samples using FT-ICR MS at the National High Magnetic Analytical Facility in Florida. The ultrahigh resolution capabilities of FT-ICR allows absolute elemental composition of each mass detected to be determined. We suggest FT-ICR MS analysis of approximately 10 -15 rainwater samples selected from our suite of preserved rainwater samples which were collected during the present project. That information would then be analyzed in conjunction with our Molecular Marker Database of Atmospheric Organic Compounds.

Bioavailable DOM Compounds. An equivalent amount of total DOC and DN was used from the rainwater tested from both Camden and Pinelands by aquatic microorganisms. However, the effects differed: the biomass production of the aquatic bacteria was three times higher when growing on DOM from the Pinelands rainwater compared to the Camden rainwater. ESI-MS analysis demonstrated that approximately half of the compounds in rainwater from both sites were utilized (bioavailable). The chemical characteristics of these compounds indicates that a similar percent of compounds with acidic and with basic type function groups were utilized, although initially there were considerably more compounds in the rainwater with basic functional groups. Preliminary matches were made between compounds (m/z) that were bioavailable and compounds in the Molecular Marker Database. To date, over 25 matches have been made and include potentially both anthropogenic and biogenically produced compounds. Further chemical characterization is required to confirm identification of compounds, as noted above.

Overall. Significant new knowledge was developed by this project on the molecular chemical composition, sources, and ecosystem effects of atmospheric DOM. We now know that there is a large number of DOM compounds in rainwater and that their molecular weight is relatively small compared to much of the DOM in surface waters. We know which masses occur with high frequency, which show seasonal patterns, have some information on differences in DOM chemical composition as a function of storm trajectory, and information on which masses are bioavailable. Significant new analytical capabilities were developed during the project which can be used to further compound identification. A critical database of Molecular Markers of Atmospheric Organic Compounds was developed. Next steps in absolute compound identification for the suite of organic masses of interest were identified. Three post-docs, three graduate students and numerous undergraduate students participated in and benefited from this project. Over a dozen presentations were made at local, national and international scientific meetings and several manuscripts are in preparation. The project also lead to numerous new collaborations. This includes collaborations with Dr. Barbara Turpin (Rutgers Univ., Environmental Sciences) in studies of SOA formation using ESI-MS. Overall, the project produced significant new knowledge on atmospheric organic compounds in wet deposition to address both science and management needs.

Chemical Composition and Bioavailability of Dissolved Organic Nitrogen in Atmospheric Wet Deposition from Urban and Rural New Jersey Sites

Background and Significance

Natural and anthropogenic N compounds are emitted to the atmosphere as oxides of N (NO_X, that is NO, NO2), ammonia, and organic-N (e.g., Graedel et al., 1986, Galloway et al., 1995, Finalyson-Pitts and Pitts, 1985, 2000). Anthropogenic activities are the main source of inorganic-N (e.g., NO_X and ammonia) to the atmosphere. For example, in North America the dominant sources of the NO_X compounds are from fossil fuel combustion in motor vehicles and electrical power generators, and from the manufacture of nitric acid or nitrated substances (Council of the Commission for Environmental Cooperation, 1997; U.S. EPA 1996, 1997; NARSTO 2000). These and other anthropogenic sources have increased the inorganic-N inputs to the atmosphere by 300% during the past 20 years and resulted in increased N deposition to many terrestrial and aquatic ecosystems (Fisher et al., 1988, Galloway et al., 1995; Munger et al., 1996, 1998). Shifts in plant and algal species composition, increased productivity, decreased species diversity, and changes in food web structure can result from these increased N inputs (e.g., Vitousek and Howarth, 1991; National Research Council, 1993; Wedin and Tilman, 1996; Paerl et al. 1990*)*.

To date, inorganic-N has been the focus of most measurements (including NJ) and models of N emissions to the atmosphere. Similarly, inorganic-N has been highlighted as part of atmospheric deposition studies of N (wet and dry) and has been the dominant N form studied in relation to effects on ecosystems (e.g., NAPAP 1991; NADP/NTN 1996; Wedin and Tilman 1996; Paerl et al. 1990). However, data from a wide range of locations (urban, rural, and remote) demonstrate that organic-N comprises an additional important component of N in rainwater (Table 1). Hints of the importance of organic-N in precipitation trace back to studies as early as the mid-1800's in France and England (reviewed by Mazurek and Simoneit 1986). More recent studies demonstrate that atmospheric organic-N typically accounts for 20%-70% of the total N annually (Table 1). Neither the chemical forms, sources or the effects of this organic-N on ecosystems have been adequately identified and measured for any system.

In addition to the N content of organic matter, polar organic compounds in the atmosphere are of interest for additional scientific and societal reasons, including the changing fluxes of carbonaceous matter between the atmosphere and the Earth's surface (IPCC 1996; Willey et al. 2000), impaired atmospheric visibility and poor air quality (National Research Council 1993; U.S. EPA 1996), influence on cloud properties (and climate) due to interactions with atmospheric water (e.g., Blando and Turpin 2000), deposition of acidic compounds to ecosystems, and effects on human health (e.g., Finlayson-Pitts and Pitts 1997; Hannigan et al 1994). Thus is it important to understand the full suite of organic compounds (both with and without N) in the atmosphere, including in wet deposition.

Policy Issues

As a result of the Clean Air Act, nitrogen oxide inputs to ecosystems are predicted to decrease in the US over the next 10 years (Periasepe 2000). EPA is undertaking a major, multifaceted nitrogen oxide initiative, including reducing emissions from power plants and requiring cleaner cars, trucks and buses, and lower-sulfur fuel. When fully implemented, it is projected these programs will produce a significant reduction of NOx pollution in NJ, as well as nationwide. However, as noted above, a major component of N in rainwater is dissolved organic N (DON), and it is not known how these new management approaches will affect the amount or forms of DON in atmospheric deposition. For example, 1) if DON has no links to processes producing NOx, then the amount of DON in atmospheric deposition should stay constant; however, DON would become an increasingly higher percentage of the total N deposited, 2) if some DON compounds are from secondary reactions with NOx and ozone, total DON deposition would decrease, due to a decrease in the deposition of specific DON compounds; 3) similarly, if some DON compounds are more directly linked to primary emissions and atmospheric photochemical processes involving NOx, total DON may decrease due to a decrease in the emissions and secondary production of those specific compounds, and/or 4) DON may increase in amount due to other human activities. Thus, now is a critical time to begin monitoring the amount of DON in rain, changes in the chemical composition of DON compounds in rain, and utilization of DON by organisms in the environment.

Table 1. *Percent composition of inorganic (DIN) (ammonia plus nitrate) and organic N in precipitation from various locations.* (modified from Seitzinger and Sanders, 1999)

Source/Location	DIN %	**Organic N %**
Walker Branch, TN[1]	68	**32**
Coweeta, NC[2]	53	**47**
Coastal plain, FL[3]	57	**43**
Cascade Mtns., OR[4]	32	**68**
Coastal plain, SC[5]	51	**49**
Philadelphia, PA[6]	70	**30**
Chesapeake Bay[7]	43	**57**
Rhode River, MD[8]	55	**45**
Raritan Bay, NJ[9]	62	**38**
UK[10]	79	**21**
N. Carolina[10]	79	**21**
Amazonia[10]	78	**22**
Recife, Brazil[10]	75	**25**
Bermuda[10]	41	**59**
Tahiti[10]	16	**84**
NE Atlantic[10]	33	**67**

[1] Kelly and Meagher 1986; [2] Swank and Waide 1987; [3] Riekerk 1983; [4] Grier et al. 1974, Fredriksen 1983; [5] Richter et al. 1983; [6] Seitzinger unpubl. data; [7] USEPA 1982; [8] Peterjohn and Correll 1984; Jordan et al. 1995; [9] Seitzinger, unpublished data; [10] Cornell et al. 1995.

Table 2. Summary of the major science questions, objectives and approaches for study of atmospheric dissolved organic matter deposition in New Jersey.

Science Question	Objectives of Current Study	Approach for this Study
I. What are the sources of dissolved organic N deposited in rain, and how do they differ in undisturbed versus urban areas?	A. Determine the chemical composition of dissolved organic N (DON) in rain at two relatively undisturbed and two urban sites in NJ; use that information to obtain insight into: 1) the relative magnitude of biogenic sources, anthropogenic sources, and atmospheric photochemical reactions, and 3) long-range transport versus local sources.	1. Analyze chemical composition of organic compounds in rain collected over two annual cycles in: 1) Camden and 2) the Pinelands, NJ. Seasonal patterns, meteorology and storm origin are linked to precipitation chemistry. 2. Determine dissolved organic matter compounds whose functional group composition is indicative of: 1) biogenic, 2) anthropogenic, and 3) photochemical processes. 3. Search for specific chemical molecular markers within each of the 3 source categories. This information will be used to link specific types of processes to atmospheric loadings (e.g., combustion, manufacturing processes, vehicular exhaust, landfill emissions, photochemical, natural vegetation).
II. What is the disruption to ecological systems from DON in rain, and how does that differ in relatively undisturbed and urban rain?	A. Determine how much of the DOM in rain from relatively undisturbed and urban sites is utilized by aquatic microorganisms.	1. Examine stimulation or inhibition of aquatic microbial populations due to DOM additions from rain collected at the 2 study sites to microcosms of aquatic microorganisms. Quantify total amount of DOM utilized.
	B. Determine which of the DOM compounds in rain from the undisturbed and urban sites are utilized by receiving ecosystems.	1. Determine which of the DOM compounds in rain decrease when subjected to degradation by aquatic microorganisms.
	C. Begin to relate bioavailable organic components to sources (biogenic, anthropogenic or photo-chemical)	1. Compare utilization of individual compounds with information linking specific types of processes to those chemical compounds (e.g., combustion, manufacturing processes, vehicular exhaust, landfill emissions, photochemical, natural vegetation).

Science Issues

What are the sources to the atmosphere of organic N compounds? (Table 2)

The chemical composition of dissolved organic nitrogen (DON) in rainwater has only been partially characterized (Gorzelska et al. 1997). However, the available data indicate a variety of sources, both natural and anthropogenic. Analyses of the ^{15}N content of bulk DON from a range of locations (Cornell et al., 1995) as well as variations in the C:N ratios of the bulk DON in urban sites (Seitzinger, unpublished data) indicates multiple sources. The range of different chemical compounds in the atmosphere and in precipitation also indicates a range of sources. Over 100 different organic-N containing compounds in the atmosphere have been reported, which are candidate chemicals to be included in rain DON (reviewed by Graedel et al. 1986).

From Graedel et al.'s (1986) review of the multitude of organic-N compounds in the atmosphere and from measurements of specific compounds in precipitation, we can see that they fall into three main functional classifications: 1) reduced N (e.g., nitriles, amines, amides); 2) aromatic N (N-heterocycles); and 3) oxidized N (alkyl nitrates, nitro-aromatics) (Table 3). These three classes can be used to begin to identify and quantify the major categories of sources contributing to atmospherically deposited organic-N. For example, most amines have a biogenic source, while the heterocycles (aromatic species) mostly arise from combustion processes and are present in crude oil and refined petroleum products. Nitro compounds also would be primarily from combustion sources, although indirectly, through photochemical reactions in the atmosphere between NO_X (anthropogenic origin) and ozone (anthropogenic), to form nitro aromatic compounds with the nitro group attached at the 2-carbon position (Finalyson-Pitts and Pitts, 1997 and 2000). ***Thus, the chemical composition of DON can provide critical information to identify the general categories and specific sources of atmospheric organic-N substances***.

Anthropogenic sources are likely to be an important component of DON in rainwater based on a number of lines of evidence. For example, while amines, which generally indicate a biogenic and thus partially natural source, are a consistent component in precipitation, the total amount of amines often accounts for only a small fraction of the total DON. A number of organic-N compounds with anthropogenic sources have been reported in field studies. Laboratory investigations have calculated significant wet removal rates for hydroxy organic nitrates (form of oxidized-N) from the atmosphere (Shepson et al., 1996). Other water-soluble anthropogenic organic-N compounds found as atmospheric particulate matter that are likely to be found as DON in precipitation have been reported in a critical literature review (Saxena and Hildemann, 1996). However, previous studies have generally not taken a mass balance approach for total DOC and DON in rainwater, but rather have focused on a particular compound or group of compounds. A mass balance inventory that accounts for all organic matter in rainwater is a first step toward quantifying the relative contribution of different forms, and thus different sources, of organic-N and C to atmospheric deposition. A mass balance approach is central to our analytical scheme. Our long-term goal is to quantify, to the maximum extent possible, the absolute and relative magnitude of reduced, heterocyclic and oxidized N compounds in atmospheric deposition at 2 sites in New Jersey. We aimed to identify as many individual compounds as possible and to determine chemical properties of compounds that can not be specifically identified. The N functional group scheme, as well as C and O functional groups, will be used to classify broadly the molecular indicators according to sources from biogenic (reduced-N compounds) processes and anthropogenic processes (heterocyclic and oxidized organic-N compounds). Detailed information on the chemical structure of specific compounds within each of these 3 categories will be used to further identify specific types of sources (combustion, vehicular exhaust, photochemical conversion, manufacturing, etc.) within each category.

Table 3. Summary of DON component groups, molecular compositions, and major sources.

DON Component	**Wet Deposition Field Study**	**Sources** *(Major Sources)*
Reduced Species		
Amines	Semenov *et al.*, 1967a; Gorzelka *et al.*, 1990, 1992, 1994, 1997	*Biogenic* (primary emission); animal feedlots, biological decay processes (*1,2*)
Primary amines	Likens *et al*., 1983; Mopper & Zika, 1987	
Secondary amines		
Tertiary amines		
Diphenylamine	Lunde *et al*., 1977	*Industrial*, chemical manufacturing, combustion
Amino acids (bulk)	Fonselius, 1954; Semenov *et al.*, 1967ab; Rozinoyer *et al.*, 1973; Timperly *et al.*, 1985 Mopper & Zika, 1987; Gorzelska *et al*., 1990, 1992, 1994, 1997	*Biogenic* (primary emission)
Amino acids (individual compounds)	Sidle, 1967; Mopper & Zika, 1987; Gorzelska *et al.*, 1990, 1992, 1994, 1997	*Biogenic* (primary emission)
Aromatic Species		
N-Heterocyclic compounds		*Diesel exhaust* (primary emission), tobacco smoke, coal combustion, animal waste, chemical manufacturing *(1,9)*
Nitro-PAH		*Diesel exhaust*, chemical manufacturing *(1,2);* Photochemical (secondary compound) *(2,9)*
Oxidized Species		
Amides		*Industrial, tobacco smoke*, animal waste *(1)*
Urea	Semenov *et al.*, 1967a; Timperly *et al.*, 1985	*Biogenic*, animal waste *(1)*
Nitrates		
Alkyl nitrates		*Photochemical (2-4,5)*
Hydroxy alkylnitrates		*Photochemical (2-4*)

Methods

Precipitation Collection

Rainwater was collected at an urban site and at a rural site in south-central New Jersey during June 2001 – March 2004 (Table 4). Rainwater was collected using wet-dry deposition collectors (Aerochem Metrics Model 301, Bushnell, FL). The urban collector was installed on the roof of the Rutgers University Library in Camden, NJ, at a height of ~11 m above the ground. The city of Camden is directly across the Delaware river from Philadelphia, PA and is a heavily urbanized region. The rural collector was situated at the Rutgers University NJ Pinelands research station at ~ 1 m above ground level. The Pinelands research station is located within the Lebanon State forest and the predominant vegetation type is *Pinus rigida* (Pitch Pine). Both rain collectors were fitted with stainless-steel buckets and opened only during wet-fall events. Information about storm trajectories for each rain event was obtained from the National Weather Service website (www.nws.com).

Samples were collected event-wise, and water was retrieved from the collectors within ~12 hours of each rain event to minimize microbial degradation of dissolved organic matter and consumption of inorganic nutrients. Sample temperature and pH were measured immediately after collection and samples were filtered through pre-combusted glass fiber filters (Whatman, GFF; baked for four hours at 500 ºC; then rinsed with deionized water). Rainwater was stored frozen in polypropylene screw-capped tubes until analysis. Filter blanks and de-ionized water blanks were collected to assess systematic contamination from sample handling and processing methods. Field blanks were collected by placing ~150 ml of low-carbon, deionized water (e-pure water; Barnstead, Inc.) into the collectors and then collecting the water in the same way as a rainwater sample. Field blanks were designed to assess contamination that might arise from the collector itself or from the handling of samples.

Chemical Analyses

Bulk Nutrient and Carbon Analysis

Bulk nutrients ($NO_3^- + NO_2^-$, NH_4^+, PO_4^{3-}) were measured on each rain sample with an automated nutrient analyzer and standard colorimetric methods (Lachat, Inc; QuickChem methods, NH_4^+: 31-107-06-1-A; $NO_3^- + NO_2^-$: 31-107-04-1-A; PO_4^{3-}: 31-107-04-1-A). Dissolved organic carbon (DOC) was measured by high-temperature catalytic oxidation with a Shimadzu TOC 5000A analyzer according to the method outlined in Sharp et al. (1993). Dissolved organic nitrogen was determined as the difference between total dissolved nitrogen measured with an Antek 7000 TN Analyzer (Seitzinger and Sanders, 1999) and dissolved inorganic nitrogen ($NO_3^- + NO_2^-$, and NH_4^+). Calibrations were performed using KHP standards for DOC and urea and nitrate standards for TDN. Reference materials provided by Dr. Dennis Hansell (Univ. Miami, FL) were included in every DOC run as an additional check on analytical performance. Our laboratory also participated in a group intercalibration exercise for the analysis of TDN headed by Dr. Jonathan Sharp (Univ. Delaware) (Sharp et al., 2004).

Season	Months	# Rain Events	TABLE 4. Sample Collection Dates													
Camden																
Spring	March - May	0														
Summer	June - August	6	6/2/2001	6/12/2001	8/11/2001	8/12/2001	8/13/2001	8/14/2001								
Fall	September-November	3	10/15/2001	10/17/2001	11/20/2001											
Winter	December-February	7	12/9/2001	12/14/2001	12/18/2001	1/8/2002	1/21/2002	1/25/2002	2/11/2002							
Spring	March - May	8	3/3/2002	3/14/2002	3/18/2002	3/21/2002	3/27/2002	4/1/2002	4/26/2002	4/28/2002	5/2/2002	5/9/2002	5/14/2002			
Summer	June - August	7	6/6/2002	6/7/2002	6/14/2002	6/18/2002	6/20/2002	7/10/2002	8/29/2002							
Fall	September-November	14	9/16/2002	10/5/2002	10/11/2002	10/12/2002	10/16/2002	10/26/2002	10/30/2002	11/6/2002	11/12/2002	11/13/2002	11/17/2002	11/18/2002	11/22/2002	11/27/2002
Winter	December-February	7	12/6/2002	12/12/2002	12/14/2002	1/30/2003	2/4/2003	2/8/2003	2/19/2003							
Spring	March - May	9	3/3/2003	3/7/2003	3/21/2003	3/27/2003	4/8/2003	4/10/2003	4/12/2003	5/6/2003	5/8/2003					
Summer	June - August	1	6/4/2003													
Fall	September-November	2	9/13/2003	9/19/2003												
Winter	December-February	1	12/8/2003													
Spring	March - May	1	3/19/2004													
Pinelands																
Spring	March - May	3	5/21/2001	5/22/2001	5/30/2001											
Summer	June - August	9	6/12/2001	7/5/2001	7/18/2001	7/27/2001	8/11/2001	8/13/2001	8/13/2001	8/14/2001	8/28/2001					
Fall	September-November	6	9/6/2001	9/11/2001	9/14/2001	9/21/2001	9/25/2001	11/26/2001								
Winter	December-February	9	12/9/2001	12/11/2001	12/18/2001	1/7/2002	1/8/2002	1/11/2002	1/24/2002	1/31/2002	2/28/2002					
Spring	March - May	10	3/3/2002	3/14/2002	3/18/2002	3/21/2002	3/27/2002	4/2/2002	4/25/2002	5/2/2002	5/13/2002	5/28/2002				
Summer	June - August	10	6/6/2002	6/7/2002	6/13/2002	6/19/2002	6/28/2002	7/10/2002	7/20/2002	7/24/2002	8/23/2002	8/29/2002				
Fall	September-November	14	9/16/2002	9/27/2002	10/11/2002	10/12/2002	10/16/2002	10/26/2002	10/30/2002	11/6/2002	11/12/2002	11/13/2002	11/17/2002	11/18/2002	11/22/2002	11/27/2002
Winter	December - February	7	12/6/2002	12/12/2002	12/14/2002	1/30/2003	2/4/2003	2/8/2003	2/19/2003							
Spring	March - May	10	3/3/2003	3/5/2003	3/7/2003	3/21/2003	3/27/2003	4/8/2003	4/10/2003	4/12/2003	5/6/2003	5/8/2003				
Summer	June - August	1	6/4/2003													
Fall	September-November	2	9/13/2003	9/19/2003												
Winter	December-February	1	12/8/2003													
Spring	March - May	1	3/19/2004													

Molecular-level Analysis

Atmospheric Pressure Electrospray Ionization Mass Spectrometry (ESI MS). Rainwater samples (92 samples) collected over 2 annual cycles plus 8 target samples from specific storm trajectories from the Camden and Pinelands sites were analyzed for molecular level chemical characterization by ESI-MS. Dissolved organic matter (DOM) was analyzed using an Agilent 1100 Liquid Chromatograph/Mass Spectrometer (LC/MS) with an autosampler, quadrupole mass spectrometer, and an electrospray ionization source. The autosampler injects samples and standard solutions (20 l) from individual vials into the system; samples flow directly to the ESI source region of the mass spectrometer. The mobile phase (pH 3.5) was 50:50 v/v methanol/water (0.05% formic acid in E-pure water) with a 0.22 ml min^{-1} flow rate. The ESI-MS measurements were conducted in both ESI-positive and ESI-negative ionization modes over the range 50 to 1000 amu (fragmentor voltage: 40 V; capillary voltage: 3 kV; N_2 drying gas: 350 ºC @ 10 L min^{-1}, 25 psig) (Seitzinger et al., 2003). The mass spectra were recorded on Agilent software (ChemStation version A.07.01) and then imported into MSAccess and MSExcel (Microsoft, Inc.) for statistical analysis and interpretation. The ESI-MS generally does not fragment compounds because the ionization process uses a low fragmentor voltage; thus, it provides molecular weight information as a molecular ion peak at an individual mass-to-charge ratio, m/z (Cole, 1997).

The two different ionization modes (ESI-positive and ESI-negative) provide basic chemical information about the nature of the functional groups present on a compound. Depending upon the charge applied to the spray capillary in the ionization chamber, positive or negative ions will be accelerated toward the inlet capillary and detected by the mass spectrometer. Under ESI-positive conditions, those compounds with electron acceptor functionality (predominantly basic-type compounds; e.g, alkanes, aromatics and nitrogen heterocycles) acquire a net positive charge and are accelerated toward the mass spectrometer. Under ESI-negative conditions, electron-donor compounds (predominantly acidic compounds; e.g., carboxylic acids, amino acids, and compounds with amide groups) become negatively charged and are likewise electrostatically directed to the detector.

Each rain event was analyzed using six replicate injections in both ESI-positive and ESI-negative ionization modes to establish a strong statistical basis for interpreting the mass-to-charge ratio (m/z) and ion abundances generated by the mass spectrometer. Samples were analyzed over the mass range of 50-1000 amu, as previous rainwater analyses indicate that very few dissolved compounds occur in the higher mass range (1000-3000 amu; Seitzinger et al., 2003).

N composition. Characterization of the potential number of N atoms in each compound was obtained by applying the “N rule.” If a compound has an odd mass it definitely has a N atom in its structure, and contains an odd number of N atoms (1,3,5 etc.). If a compound has an even mass, it has either no N or an even number of N atoms in its structure (0, 2, 4 etc.).

Advanced Chemical Characterization. The above approach provides detailed compound molecular weight information (unit mass resolution), acid/base functional group information, and insight into the number of N atoms (odd/even) in the structure. We can begin to identify potential compounds and their concentration based on the combined information from those analyses, the Molecular Marker Database of Atmospheric Organic Compounds that we developed (see below), and the analysis of authentic standards of atmospheric significance. However, many compounds can potentially have these sample characteristics. In the current project we began development of two additional methods to enhance the ESI-MS analyses: 1) determining response factors for compounds across a range of instrument operating conditions and 2) combining high pressure liquid chromatography (HPLC) with the ESI-MS.

Authentic standards were analyzed across a range of ESI-MS fragmentor voltages (FV) (40-120 V) to determine how their ESI-MS response factors varied as a function of FV. The standard compounds chosen were those that showed potential matches with compounds in the rainwater samples based on their m/z and positive/negative mode detection. In addition, rainwater samples were spiked with these standard compounds and analyzed across the range of FV to determine matrix effects. Selected rainwater samples from both Camden and the Pinelands were analyzed as well across the same FV and the response of the m/z in the sample was compared to the matching standard. Spiked and unspiked rainwater samples were also analyzed using HPLC (C18 column). The retention times of sample m/z's were compared with the potential matching standard m/z for further identification.

Sample Processing. The ESI-MS output for each sample was processed as follows to arrive at a final data set for analysis and interpretation. The mean ion abundance (mass-to-charge ratio rounded to nearest integer ± one standard deviation: S.D.) for replicate injections of each sample was calculated for rainwater samples and instrument blanks. Each ion with an abundance statistically different from zero at the 0.05 level (t-test; (Sokal and Rohlf, 1981) is retained. The rainwater samples are then corrected for procedural blanks by subtracting the ion abundance of any peak found in the blank from the abundance of any peak at the same m/z in a sample. The standard deviation of the corrected ion abundance for each peak was calculated using the standard propagation of error equations (Bevington and Robinson, 1992). The final data set for a rainwater sample includes the average ion abundance and the standard deviation for each blank-corrected ion with an ion abundance that is statistically different from zero. Because of the generally increased variability in detecting compounds with ion abundances <500, only ions with abundances statistically greater than 500 (t-test, $\alpha = 0.05$) are interpreted in the final data set.

Inorganic ion detection by ESI-MS. ESI-MS detects organic compounds as well as inorganic compounds. This is demonstrated by detection of nitrate standards and by m/z 62 (HNO_3- minus H) in our rainwater samples in the ESI negative ionization mode. There are numerous inorganic ions that may be present in the rainwater samples. As the project developed, it appeared that the ESI-MS analysis was detecting more inorganic ions than originally anticipated. Therefore, we began to develop methods to distinguish between organic and inorganic ions (Hartnett et al. in prep.). Identification of which compounds are inorganic and which are organic is primarily through comparison of ESI-MS results of rainwater samples before and after UV oxidation (UV oxidation results in oxidation of DOC to CO_2). Less than 25% of the total peaks (non-UV oxidized samples) are inorganic compounds. More inorganic species are present in the negative mode than in the positive mode. It was not possible to analyze all samples before and after UV oxidation as the analysis of UV oxidized samples would have doubled the number of samples to be analyzed by ESI-MS. This was not in the original proposal or the budget (neither the instrument nor data interpretation costs). Therefore, in this report we have not subtracted m/z detected after UV oxidation.

Database of DOM Compounds in New Jersey Rainwater. A database was developed of ions detected in rainwater samples collected over the project period from the Camden and Pinelands sites. The database contains information on the collection date and location for each sample, each m/z detected in a rainwater sample, the ion abundance of each m/z, and the ionization mode (positive or negative) in which it was detected. This database also contains information on storm trajectory, precipitation volume, and bulk chemical properties for each sample.

Standards of Atmospheric Organic Compounds. Authentic standards of approximately 80 target marker organic compounds were obtained, analyzed by ESI-MS, and response factors determined. A comparison of the ESI-MS response to these standards with the detection of the same mass and mode (+/-) response from rain samples was used to make a preliminary evaluation of what specific compounds are in the rain samples, as well as in some cases what the concentration is of these compounds.

Molecular Marker Database of Atmospheric Organic Compounds

A major effort on this project was devoted to the development of a Molecular Marker Database of Atmospheric Organic Compounds that have been reported in atmospheric samples in the literature. The database contains information on compound name, structure, CAS number, molecular weight, solubility, known sources, and literature citation, etc.. We used this database for initial interpretation of compounds in the rain samples from our study sites, for targeting compounds to obtain for additional authentic standards, and for interpretation of the bioavailability data. This molecular database is key to identifying and quantifying sources of DOM in rainwater.

Determination of Bioavailable Compounds in Rainwater

The term “bioavailable” DOM, as used in this study, refers to that portion of the DOM that was utilized by the microbial community within the 14-day incubation period, under the experiment conditions. Our bioavailability experiments are similar to those that we have developed and used previously to examine utilization and effects on aquatic organisms of DOM inputs from a variety of sources (rainwater, urban runoff, agricultural runoff, forest runoff, etc.) (Seitzinger and Sanders 1997; Seitzinger and Sanders 1999; Seitzinger et al. 2002 and 2005). The major pathway by which DOM is first incorporated into the biological cycle in aquatic ecosystems is through bacteria. Bacteria can utilize the DOM for growth (increase in biomass) or mineralized it to DIN (ammonia) and CO_2.

Microcosm Incubations

Select rainwater samples collected during Spring (Mar-May) 2002 were chosen based on their sampling location (i.e. urban or rural), storm trajectory (i.e. W-SW component), and DOC concentrations (i.e. >100 µM-C). The samples were subsequently combined for a Camden (urban) and a Pinelands (rural) composite. The composites were amended with inorganic phosphate (final incubation concentration 3.3 µM PO_4^{3-}) to avoid nutrient limitation. Forty ml of each composite was dispensed into 50 ml amber glass flasks for the different treatments. Two flasks from each site were inoculated with a natural bacterial assemblage of bacteria from a nearby pond (Weston Mills Pond) (10ml to 250ml rainwater composite). The bacterial innoculum was sonicated briefly before use to eliminate protozoa (Seitzinger and Sanders 1997). Controls to check for non-biological changes in DOM consisted of rainwater composites, not inoculated with bacteria, which were autoclaved/sterilized for 25minutes at 121°C. A de-ionized water control with the bacterial inoculate was also run to monitor for contamination during the incubation and sampling. The flasks were wrapped in aluminum foil to eliminate light, placed on a stirrer table, and allowed to incubate for 14 days at room temperature (approximately 21 ºC). Time-series samples were collected from each flask on days 1, 3, 7 and 14.

Bacterial production was determined in each sample collected from the microcosm experiment using the ^{3}H-leucine uptake method (Smith and Azam 1992 as detailed in Seitzinger et al. 2002). All samples were analyzed for dissolved inorganic N (DIN: ammonium, nitrite and nitrate), dissolved inorganic phosphate, bulk DOC, and bulk DON using the methods described above for rainwater analysis. All glassware used throughout the study was cleaned by acid washing and heating to 500^{o}C for at least 4 hours.

The total quantity of DON and DOC utilized (bioavailable component) was determined based on the difference between the amount of total DON and DOC at the beginning of the experiment and the amount remaining over time. Similarly, N and P mineralization from the DOM were quantified based on differences in concentration of ammonium, nitrate plus nitrite, and phosphate over time.

Time series samples from the bioavailability experiment were analyzed for molecular level DOM chemical composition by ESI-MS as described above for rainwater samples. Because of the generally increased variability in detecting compounds with ion abundances <200, only m/z with ion abundances >200 were included in the final data set. We determined which of the organic compounds are refractory (not utilized by the microbes) and which are biologically available (utilized) and thus potentially contributing to changes in ecosystems, by comparing the masses and their abundances in rainwater at the beginning of the experiment and at the end of the experiment after bacterial degradation.

We used our Molecular Marker Database of Atmospheric Organic Compounds to make tentative matches with m/z and positive/negative ionization detection of bioavailable m/z identified in the rainwater bioavailability experiment. This information was used to determine the potential contribution of anthropogenic versus biogenic/natural compounds to the bioavailable pool of DOM in rainwater.

Results and Discussion

Two major questions that this project aimed to address were:

What is the chemical composition of total dissolved N, both inorganic and organic nitrogen, in atmospheric deposition in 1 urban and 1 relatively undisturbed site in New Jersey?

How can the chemical composition of dissolved organic matter provide information to identify the general categories and specific source-types of atmospheric organic substances?

The predominant storm trajectory of the rain events sampled during the June 2001 –May 2003 period was from a southwesterly direction with less than 10% of samples from the E or N direction (Table 5). The amount of rain in each rain event varied considerably, and ranged from approximately 100 ml to over 3 liters (diameter of collector 28.6 cm). During Year 3 we were able to obtain 8 samples from an easterly or NW direction (Table 6).

Bulk Nutrient and Carbon Analysis

Inorganic N (ammonium, nitrate plus nitrite), phosphate, DON, DOC exhibited a general seasonal trend of higher concentrations in summer and lower concentrations in winter (Fig. 1). There were no strong differences in concentration between the Camden and Pinelands sites in these parameters. pH also exhibited a seasonal pattern, but with lower pH in summer and higher in fall through spring (Fig. 1). There appeared to be an overall trend of increasing pH over the 24 month period.

The NADP program collected ammonium, nitrate and pH data at two locations in New Jersey (Washington Crossing site ID NJ99 and Edwin Forsythe National Wildlife Refuge ID site NJ00) over the same project period (http://nadp.sws.uiuc.edu/). The latter location is a coastal site. The NADP collection methods differ in that samples are collected approximately monthly, while we collect samples on an event (daily) basis. Thus the data are not directly comparable. Ammonium concentrations ranged from 4-36 µM and 7-81 µM at the Forsythe and Washington Crossing locations, respectively. Nitrate concentrations ranged from 5-33 µM and 9-104 µM at the Forsythe and Washington Crossing locations, respectively. These ranges, particularly for Washington Crossing, are similar to those we measured at Camden and Pinelands. pH ranged from 4.2 to 5.0 and 3.7 to 4.8, at the Forsythe and Washington Crossing locations, respectively. This is also similar to pH at our study sites, although at times lower values were measured at the Pinelands site.

Table 5. Volume, temperature, storm trajectory and pH of samples collected at Camden and Pinelands sites

CAMDEN

Collection Date	Volume Collected (ml)	Rain Temp (°C)	Rain (cm)	Storm Traj	pH
06/02/01	2815	22	4.4	WSW	3.7
06/12/01	874	23	1.2	WNW	3.9
08/11/01	1715	27	2.6	WSW	2.76
08/12/01	189	25	0.2	WSW	3.7
08/13/01	616	28	0.8	WSW	3.7
08/14/01	654	27	0.9	W	
10/15/01	505	21	0.7	SSW	4.3
10/17/01	82	17	0.2	SSW	4.1
11/20/01	284	14	0.4	SSW	4.2
12/09/01	1630	10	2.4	SW	4.3
12/14/01	595	16	0.8	WSW	3.9
12/18/01	726	13	1.0	WSW	4.5
01/08/02	3140	ice	4.4	W	4.4
01/21/02	1052	snow	1.5	SW	4.7
01/25/02	905	8	1.4	WSW	4.3
02/11/02	392	10	0.6	SW	4.6
03/03/02	1815	16	3.0	SW	4.9
03/14/02	520	25	0.8	WSW	4.5
03/18/02	1418	snow\ice	2.2	W	4.8
03/21/02	2037	10	3.2	SW	4.8
03/27/02	1307	10	2.1	SW	4.6
04/01/02	1004	13	1.4	W	4.4
04/26/02	520	18	0.9	WSW	4.1
04/28/02	1835	20	3.0	WSW	4.6
05/02/02	615	22	1.0	W	4.6
05/09/02	350	17	0.6	W	3.6
05/14/02	1945	15	3.0	SW	4
06/06/02	102	38	0.2	W	3.8
06/07/02	2230	18	3.2	WSW	4.5
06/14/02	1110	19	2.8	WSW	4.4
06/18/02	237	34	0.3	W	4.1
06/20/02	1760	25	2.6	WSW	4
07/10/02		25	2.0	WNW	4
08/29/02	1695	17	2.6	SW	4.8
09/16/02	226			WSW	4
10/05/02	238	21	0.4	W	4.1
10/11/02	2365	15	3.8	SW	4.46
10/12/02	1737	18	3.0	SW	5.16
10/16/02	1195	17	2.0	S	4.66
10/26/02	1959	15	3.0	WSW	4.784
10/30/02	1630	5	2.4	WSW	4.482
11/06/02	1265	12	2.0	WSW	4.795
11/12/02	1060	13	2.0	SW	4.782
11/13/02	1470	11	2.2	SSW	4.903
11/17/02	2555	6	3.8	SSW	4.474
11/18/02	955	10	1.6	SSW	4.635
11/22/02	435	13	0.8	SSW	4.185
11/27/02	480	5	0.8	WSW	2.26
12/06/02	1410	snow ~6-		WSW	5.174
12/12/02	2325	5	2.8	SW	4.247
12/14/02	1150	6	2.0	SW	4.378
01/30/03	430	snow\ice		W	4.213
02/04/03	555	10	0.6	WSW	4.552
02/08/03	1195	snow		SW	4.79
02/19/03	1170	snow		SW	4.959
03/03/03	1765	frozen		SW	4.599
03/07/03	1420	frozen		WSW	4.115
03/21/03	2507	13		SW	5.05
03/27/03	480	6	0.6	SW	4.385
04/08/03	920	3.5	1.4	SW	5.155
04/10/03	730	4	1.2	WSW	4.18
04/12/03	1570	10	2.7	SE	4.28
05/06/03	330	12	0.6	W	4.2
05/08/03	542	16	0.8	W	4.6
06/04/03	2545	14	4.0	SSE	4.6
09/19/03	1503	28	2.0	SE	4.8

PINELANDS

Collection Date	Volume Collected (ml)	Rain Temp (°C)	Rain (cm)	Storm Traj	pH
05/21/01	197	16	0.4	W	4.4
05/22/01	665	21	1.4	WSW	3.7
05/30/01	609	14	1.2	WSW	3.5
06/12/01	450	21	0.8	ENE	3.4
07/05/01	1790	20	3.55	W	3.7
07/18/01	400	21	0.65	WNW	3.4
07/27/01	67	N/A	0.05	WSW	3.6
08/11/01	220	27		WSW	
08/13/01	2145		4.7	WSW	3.9
08/13/01	136	25	0.2	WSW	3.6
08/14/01	605	24	1.2	WSW	3.4
08/28/01	370	23	0.6	WSW	3.7
09/06/01	640	26	0.8	WNW	3.7
09/11/01	245	22	0.4	WNW	4.0
09/14/01	840		1.6	W	3.7
09/21/01	985	22	1.9	SSW	4.2
09/25/01	1940	20	4.2	SSW	4.5
11/26/01	993	18	2	SW	4.2
12/09/01	1821	9	3.2	SW	4.1
12/11/01	150	18	0.2	SSW	4.4
12/18/01	705	9	1.6	WSW	
01/07/02	1500	9	2.6	WNW	4.1
01/08/02	327	snow	0.6	W	4.0
01/11/02	730	13	1.3	SSW	3.9
01/24/02	610	13	1	SW	3.8
01/31/02	260		0.6	WSW	3.6
02/28/02	150	ice	0.2	WSW	3.9
03/13/02	352	14	0.8	SW	3.8
03/18/02	1855	2	1.75	WSW	4.3
03/21/02	1695	11	2.25	SW	3.8
03/26/02	435	6	1	SW	3.4
03/27/02	1230	7	1.6	SW	3.8
04/10/02	530	13	1	SW	4.1
04/25/02	565	7	0.8	SW	4.3
05/02/02	1250	14	2	W	4.2
05/13/02	2240	13	4	SW	4.3
05/28/02	2540	23	4.3	SW	4.3
06/06/02	1515	23	3	W	4.1
06/07/02	2945	15	5.4	WSW	4.4
06/13/02	700	13	1.4	WSW	4.3
06/19/02	1130	20	2	WSW to W	4.2
06/28/02	1105	21	2.1	W	4.3
07/10/02	680	22	1.2	WNW	3.8
07/20/02	1175	22	2.1	WNW	3.7
07/24/02	130	20	0.3	WNW	3.7
08/23/02	330	23	0.6	NNW	3.7
09/16/02	92	24	0.03	WSW	4.0
09/27/02	1910	24	3.6	SW	4.7
10/11/02	3085	16	5.6	SW	5.2
10/12/02	1255	20	2.4	SW	5.2
10/16/02	2195	15	4	S	4.9
10/26/02	2141	15	4.2	WSW	4.8
10/30/02	1325	7	24	WSW	4.5
11/06/02	1520	13	2.6	WSW	4.9
11/12/02	805	12	1.6	SW	4.4
11/13/02	1518	10	2.8	SSW	4.7
11/17/02	2465	7	4.2	SSW	4.7
11/18/02	1013	9	2	SSW	4.6
11/22/02	950	14	1.8	SSW	4.6
11/27/02	310	5	0.6	WSW	4.0
12/06/02	1460	snow		WSW	5.1
12/12/02	2257	4	2	SW	4.6
02/08/03	1065	snow		SW	4.8
02/19/03	1647			SW	5.0
03/03/03	1475	FREEZING	0.4	SW	4.8
03/05/03	420	13	1	SW	4.9
03/07/03	1135	ICE		WSW	4.7
03/21/03	1500	14	0.6	SW	4.8
03/27/03	465	3	0.6	SW	
04/08/03	1245	snow		SW	4.9
04/10/03	1055	4	1	WSW	4.8
04/10/03	1055	4	1	WSW	4.8
04/12/03	875	10	1.5	SE	4.5
05/06/03	155	13	1	W	4.5
05/08/03	715	16	1.2	W to NW	4.6

Table 6. Volume, temperature, storm trajectory and bulk properties of samples collected from other storm trajectories

CAMDEN

Collection Date	Volume Collected (ml)	Rain Temp (^{o}C)	Rain (cm)	Storm Traj	pH	NO3_µM	NH4 (µM)	DOC (µM)	TDN (µM)
9/13/2003	1127	26.5	1.7	SE	5.3	7.4	8.0	34.0	15.0
9/19/2003	1503	28.0	2.0	SE	4.8	10.1	13.2	66.1	21.9
12/8/2003	1455	snow ~15"		SW to NNE	5.0	13.0	13.8	35.4	25.9
3/19/2004	1293	snow+ice		W then NW	4.5	38.4	21.8	56.8	59.4

PINELANDS

Collection Date	Volume Collected (ml)	Rain Temp (^{o}C)	Rain (cm)	Storm Traj	pH	NO3_µM	NH4 (µM)	DOC (µM)	TDN (µM)
9/13/2003	1373	27.0	1.6	SE TO SSE	5.2	4.8	1.8	23.6	8.5
9/19/2003	1565		1.4	SE	4.8	10.7	11.2	61.4	23.7
12/8/2003	1665	snow ~15"		SW to NNE	4.8	12.8	5.3	28.2	20.3
3/19/2004	1317	snow+ice		W then NW	4.4	30.4	17.0	37.2	47.9

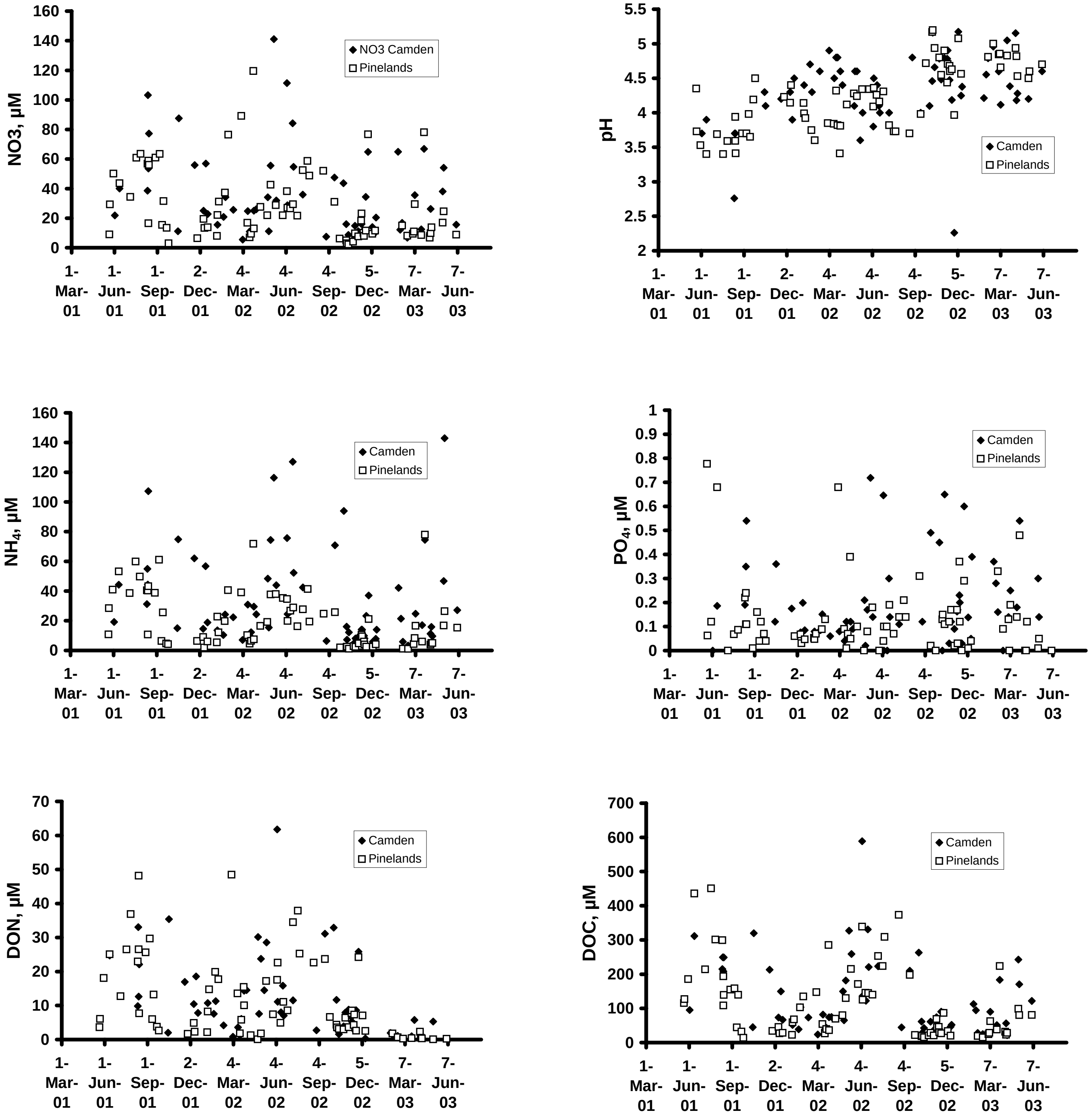


Figure 1. Inorganic N and P, DON, DOC, and pH measured in event based rainwater collections at the Camden and Pinelands study sites over the 24 month sampling period.

Chemical Characterization of DOM

Data processing

Each sample analyzed by ESI-MS provided 11,400 data points (950 potential masses per mode, 2 modes (+ and -), 6 replicate injections per sample). Processing of this data into a format that could be analyzed statistically and interpreted was initially very time intensive. When we began this project it took approximately 5 days for ESI-MS analysis of 12 samples plus associated blanks and standards (high/low mass ranges, +/- modes). Following ESI-MS analysis it then took approximately 12 person-weeks to do the initial data processing of those samples (before any data interpretation could begin). As part of this NJDEP project we devoted a considerable effort to developing computer programs to reduce the time needed for initial formatting and processing of data. It still takes approximately 5 days to chemically analyze 12 samples with associated blanks and standards. However, it now takes approximately 8 days to complete the initial processing of that data (compared to 12 weeks or 60 days when we began this project). This is a major advancement.

Ninety-two (92) rainwater samples collected between June 2001 and May 2003 plus eight addition samples from specific storm trajectories (June 2003-March 2004) were analyzed by ESI-MS (Table 5). This included 48 samples from Camden and 52 samples from the Pinelands site. All ESI-MS data were compiled into a database for data interpretation.

A large number of DOM compounds occur in rainwater at the two New Jersey sites. Overall, 832 unique m/z were detected by ESI-MS in the 92 precipitation samples collected over two annual cycles. The database contains *over 1 million data points* (1,048,800). In addition, approximately *another 1.5 million data points* were generated by ESI-MS in this project for standards, blanks, UV oxidized samples, and sample spikes. This is the most extensive molecular level database available for rainwater chemical composition and can provide the basis for significant new insight into the chemical properties and sources of a large suite of DOM compounds in rainwater.

Some compounds occurred at high or intermediate frequency, while others occurred in very few samples. In order to focus our efforts on the interpretation of this large database, we chose to remove from further analysis the large number of m/z (333) that occurred in less than 20% of the samples in each season at both sites. These m/z's often had low abundance. Their low frequency of occurrence and low abundance indicates that they are not necessarily an important component of the DOM. Therefore, we removed these m/z's from further interpretation in the remainder of the project. We call these Category I compounds. This left 499 unique m/z's in the database for subsequent interpretation. We refer to this as the C&P ESI-MS rainwater database.

Interpretation of DOM Composition in New Jersey Rainwater

The DOM compounds are relatively low molecular weight, and ranged from approximately 50 to 620 amu. (The m/z equals molecular weight+1 for positive mode detection and molecular weight-1 for negative mode detection assuming singly charged compounds. An example of an ESI-MS mass spectra for rainwater from the Camden and Pinelands site is shown in Figure 2. The molecular weight distribution of m/z detected in Camden and Pinelands rainwater is similar to the molecular weight distribution of atmospheric organic compounds reported in the literature, based on our Molecular Marker Database of Atmospheric Organic Compounds (Figure 3).

A second level of insight into the chemical structure of each compound is provided by knowing whether a compound is detected in the negative and/or positive ESI ionization mode. Compounds detected in the positive ESI mode (e.g. polyaromatic hydrocarbons, amines, amides) are expected to have functional groups that are electron donating (or proton accepting) and are thus compounds that are able to “acquire a positive charge”. In ESI-positive, compounds with the following basic functional groups should be detected (e.g. -CH_3, -NH_2, –OH, -OCH_3, -$N(CH_3)_2$,) (Table 7) (Seitzinger et al., 2003, and in press). Compounds detected in the negative ESI mode (e.g. amino acids, carboxylic acids, nitrate compounds, carbonitriles) should have electron withdrawing (or proton donating) functional groups and can thus acquire a negative charge. This includes compounds with –NO_2 and COOH functional groups, among others (Table 7).

We analyzed over 80 authentic standards known to occur in the atmosphere that spanned a wide range of molecular weights, chemical structures and sources. Criteria generally used to target compounds for standard development were their occurrence in our Molecular Marker Database of Atmospheric Organic Compounds and occurrence in a number of rainwater samples. An excerpt from the main database of authentic standards analyzed is provided in Table 8. Using functional group and proton donating/accepting information, we made preliminary predictions as to which ESI mode the compounds would be detected in (Table 8). Indeed, we observed that almost all compounds with proton withdrawing (electron donating) groups showed up in the positive ESI mode, and compounds with proton donating (electron accepting) groups showed up in the negative mode. There were some standard compounds (e.g. amino acids) that showed up in both modes because they were comprised of both types of functional groups. Thus, analysis of these standards has provided information critical to predicting functional group characteristics of unknown compounds in rainwater samples.

More compounds in the rainwater samples from the two sites had basic functional groups than acidic function groups, based on their ESI mode of detection. Of the four hundred ninety-nine unique m/z in the rainwater samples, 194 had acidic functional groups, while 305 had basic functional groups.

Thus, the first level insight into the chemical composition of the DOM is that it is relative small molecular weight compounds. A third of those compounds have structures with acidic functional groups (e.g., –NO_2 *and COOH) and two third have basic functional groups (e.g. -*CH_3*, -*NH_2*,–OH, -*OCH_3*, -*$N(CH_3)_2$*,).*

Table 7. Examples of functional groups and in which ionization mode (positive, negative) they are predicted to be detected.

Ionization mode	Functional Group
Positive	-OH $-CH_3$ $-NH_2$ $-OCH_3$ =O $N(CH_3)_2$ N heteroatoms
Negative	$-NO_2$ -COOH CN halides

Table 8. Examples of standard compounds of known atmospheric occurrence that were analyzed to assess a positive and/or negative response using ESI-MS. The functional groups detected by ESI-MS ionization mode are indicated.

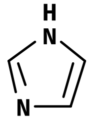
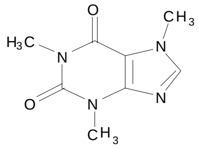
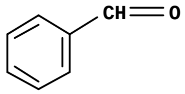
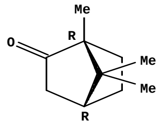
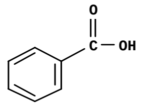
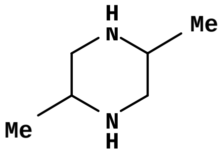
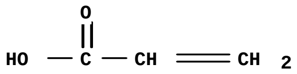
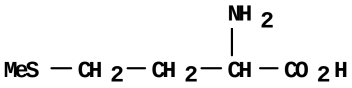
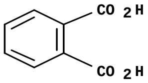
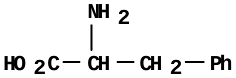
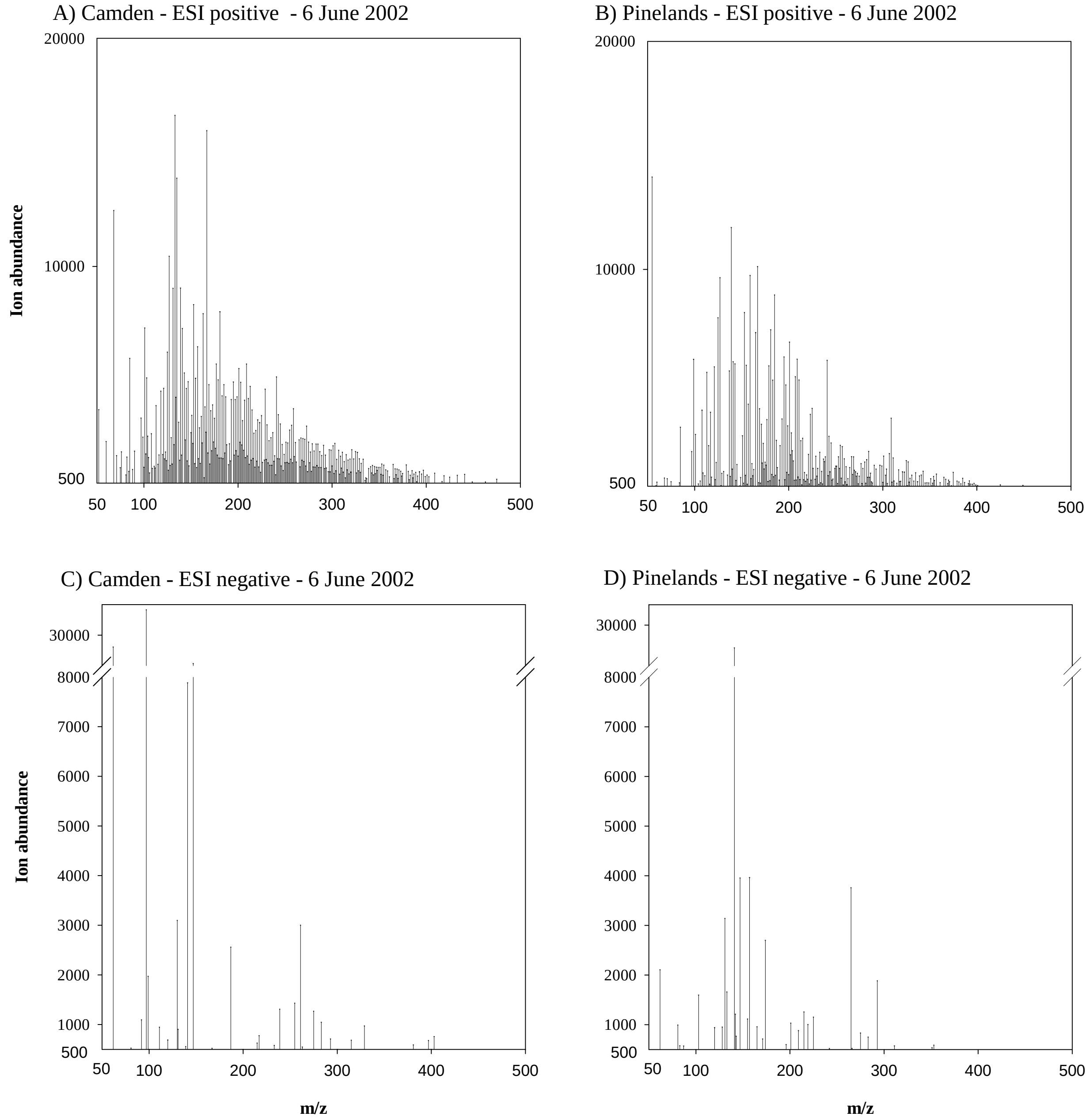
Compounds	Chemical Formula	MW	Source	Detected in ESI	Functional Groups
Imidazole		68.08	Turbine	Pos	N heterocycle
Caffeine		194.2	Refuse combustion	Pos	N heterocycle; 3CH_3; =O;
Benzaldehyde		106.12	Animal waste; automobiles	Pos	=O
Camphor		152.24	Vegetation; chemical mfr.	Pos	3CH_3; =O
Benzoic Acid		122.13	Animal waste; automobiles	Neg	COOH
1,6-Hexanediamine	$H_2N-(CH_2)_6-NH_2$	116.2	Industrial	Pos	2NH_2
2,5-Dimethylpiperazine		114.19	Diesel	Pos	N heterocycle; 2CH_3
Acrylic acid	 $HO-\overset{O}{\overset{\parallel}{C}}-CH=CH_2$	72.06	Industrial; hazardous air pollutant	Neg	COOH
Malonic acid	$HO_2C-CH_2-CO_2H$	104.06	Tobacco smoke	Neg	2COOH
Methionine	 $MeS-CH_2-CH_2-\overset{NH_2}{\overset{\vert}{CH}}-CO_2H$	149.21	Biogenic	Pos & Neg	NH_2; CH_3 (+) COOH (-)

Table 8. (continued)

Compounds	Chemical Formula	MW	Source	Detected in ESI Mode	Functional Groups
Phthalic acid		166.14	Tobacco smoke; vinyl chloride mfr	Neg	2 COOH
Phenylalanine	 $HO_2C-\overset{\overset{NH_2}{\vert}}{CH}-CH_2-Ph$	165.19	Biogenic	Pos & Neg	NH_2 (+) COOH (-)


A) Camden - ESI positive - 6 June 2002

B) Pinelands - ESI positive - 6 June 2002

C) Camden - ESI negative - 6 June 2002

D) Pinelands - ESI negative - 6 June 2002

Figure 2. Mass-to-charge ratio (m/z) and abundance of ions detected in positive and negative ionization modes with ESI-MS for rainwater collected from Camden and Pinelands sites on 6 June 2002. Note difference in Y-axis scale between positive and negative mode plots.

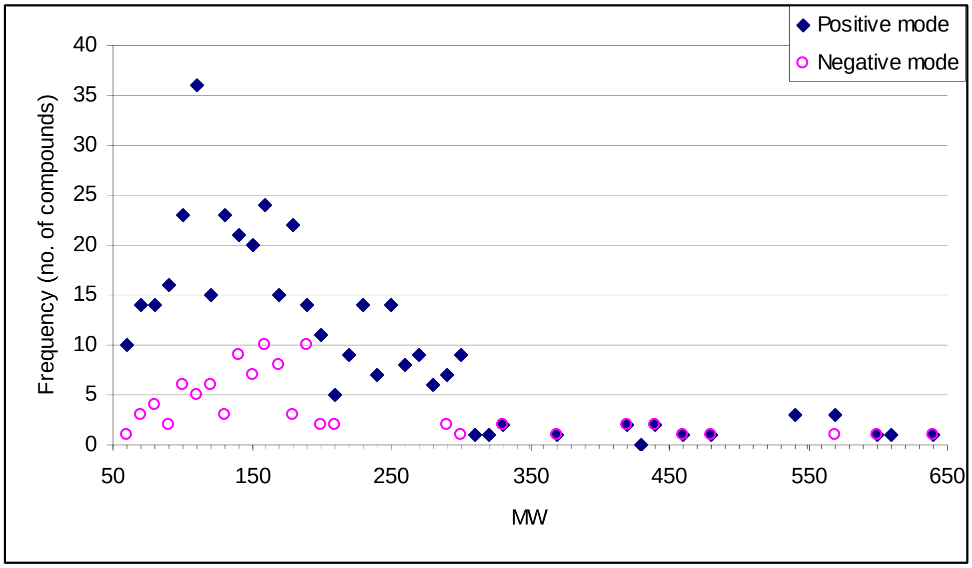


Figure 3. Molecular weight distribution of all acidic- and basic-type compounds in the Molecular Marker Database of Atmospheric Organic Compounds.

Patterns in Compound Occurrence Among Seasons and Sites

Both seasonal patterns and differences between the 2 sites were discernable in specific groups of DOM compounds. These patterns of occurrence by site and season provide insight into potential DOM source types and into which groups of compounds have potentially similar source types. The major patterns observed are summarized below. Data from the 24-month sampling period were used in the following analyses.

Groups of m/z that show (Table 9):

- **No seasonal pattern and high frequency (>70%) of occurrence at both sites (Category II).**

This is the most common pattern observed to date. Masses that occurred frequently included 95 m/z’s out of 499 (19%) which occurred in at least 64 rain samples (70% of the samples). This group of compounds showed no obvious seasonal pattern in occurrence and had generally high abundance (i.e., concentration) (Table 9).

The lack of a season pattern in occurrence suggests that these compounds are not from natural, biogenic sources, but rather from anthropogenic sources. Given the very different land use (highly urban versus forested) at these two sites, the result also suggest that many of these compounds are from sources remote to both sites, and transported to the site from longer-range atmospheric transport.

In terms of the chemical structure of these compounds, 82% of them were detected in the ESI positive ionization mode and thus had basic functional groups (e.g. -CH_3, -NH_2, –OH, -OCH_3, -$N(CH_3)_2$,). Very few m/z that met the frequency criteria were detected in the ESI negative ionization mode. Compounds detected in ESI-negative have acidic functional groups such as –COOH and -NO2. Thus, very few of the compounds in this grouping are carboxylic acids. The detection of compounds with the above basic and acidic type functional groups was confirmed by analysis of the 80 authentic standards with known chemical structures.

- **Decreasing frequency of occurrence in fall and winter at both Camden and Pineland sites (Category III)**

Masses that occurred frequently (generally >70%) in summer and spring, and that decreased in frequency in fall and winter, at both sites are termed Category III compounds (Table 9). The seasonal pattern of higher occurrence during growing seasons, with decreasing frequency in non-growing seasons, may be indicative of natural, biogenic sources. These could be local and/or longer-range sources. Of course, it is also possible that these compounds are from anthropogenic sources with a season production pattern.

- **Highest frequency of occurrence in summer at both sites (Category IV)**

Masses that occurred frequently (generally >70%) in summer and/or spring, and that decreased in frequency in fall and winter, at both sites are termed Category III and Category IV compounds (Table 9). The seasonal pattern of higher occurrence during growing seasons, with decreasing frequency in non-growing seasons, may be indicative of natural, biogenic sources. These could be local and/or longer-range sources. Of course, it is also possible that these compounds are from anthropogenic sources with a season production pattern. Secondary photochemical reactions may also be a factor in both Category III and IV masses.

- **Only occur in winter and/or fall (Category V)**

Masses that occur only in the winter, and sometimes in the fall but at lower frequency, but never in the spring or summer constitute Category V compounds. These compounds likely do not have a natural biogenic source, but rather an anthropogenic component with cold season production. Another explanation is that they are compounds that are produced year-round, but that are destroyed by photochemical processes during atmospheric transport in spring, summer and sometimes fall. If so, these compounds would probably be anthropogenic given their relatively high frequency of occurrence in winter samples.

- **No seasonal pattern at Camden but decreasing frequency in fall and winter at Pinelands (Category VI)**

One interpretation of this group is that the m/z's are comprised of different compounds in Camden compared to Pinelands, but all with the same nominal molecular weight and functional group characteristics. The lack of seasonal pattern in Camden indicates an anthropogenic origin, while the seasonal pattern in the Pinelands indicates a natural, biogenic origin.

- **High frequency occurrence in Pinelands in winter with intermediate to low frequency in other seasons and at Camden (Category VII)**
- **Never occur in the spring at either site, but occur at low frequency in other seasons (Category VIII)**

Category VII and VIII patterns, as well as numerous other patterns not discussed here, are complex. Additional chemical properties of these groups are needed to begin to understand the factors controlling the observed patterns of occurrence.

We note that while the interpretations for Category II-VIII presented above are not conclusive, they do start to provide insight into potential common source types.

The above analysis demonstrates that groups of m/z can be identified which show similar patterns of occurrence with season and with site. These patterns suggest similar source(s) for compounds in a group. Further identification of specific compounds is required to confirm the above interpretations of sources.

In the following sections we discuss the development of the Molecular Marker Database of Atmospheric Organic Compounds and its use in combination with analysis of authentic standards of atmospheric organic compounds. We provide examples of how we have used this information to gain further insight into the chemical characteristics of m/z in the rainwater from the Camden and Pinelands sites.

Table 9. Examples of masses showing patterns with season and/or site. Results are expressed as percent of samples for that site and season in which that m/z occurred.

		Camden				Pinelands			
		Season (no. of samples)				Season (no. of samples)			
		spring (12)	summer (11)	fall (10)	winter (11)	spring (11)	summer (14)	fall (11)	winter (12)
Category	**m/z**	**% occurrence**				**% occurrence**			
II	**No seasonal pattern and high frequency (>70%) of occurrence at both sites**								
	Positive Mode								
	125	100%	91%	100%	100%	100%	100%	100%	100%
	139	100%	100%	100%	100%	100%	100%	91%	100%
	141	100%	100%	100%	100%	100%	100%	100%	100%
	167	100%	100%	100%	100%	100%	100%	91%	100%
	181	100%	100%	100%	100%	100%	100%	100%	100%
	183	100%	100%	100%	91%	100%	100%	91%	100%
	197	100%	100%	100%	100%	100%	100%	91%	100%
	199	100%	100%	100%	100%	100%	100%	100%	100%
	Negative Mode								
	62	100%	100%	100%	100%	100%	100%	100%	100%
	97	100%	100%	100%	91%	100%	93%	100%	83%
	147	100%	100%	90%	100%	100%	100%	91%	100%
	165	100%	100%	100%	100%	100%	100%	100%	100%
III	**Decreasing frequency occurrence in fall and winter at both Camden and Pineland sites**								
	Positive Mode								
	162	100%	82%	50%	45%	91%	93%	45%	25%
	176	92%	73%	70%	45%	82%	79%	55%	58%
	204	75%	91%	50%	55%	73%	86%	36%	17%
	291	100%	91%	60%	64%	91%	100%	27%	67%
	325	83%	64%	60%	36%	64%	86%	45%	33%
	Negative Mode								
	129	58%	64%	40%	9%	45%	57%	9%	0%
IV	**Highest frequency of occurrence in summer at both Camden and Pinelands**								
	Negative Mode								
	213	67%	91%	50%	73%	45%	93%	36%	58%
	285	67%	82%	60%	55%	45%	93%	45%	50%
	167	67%	82%	60%	55%	55%	79%	45%	50%
	195	67%	82%	70%	45%	55%	86%	36%	25%
V	**Only occur in winter and/or fall, but not in the summer or spring at either site**								
	Negative Mode								
	299	0%	0%	30%	55%	0%	0%	18%	67%
	221	0%	0%	10%	36%	0%	0%	0%	58%
VI	**No seasonal pattern at Camden but decreasing frequency in fall and winter at Pinelands**								
	Positive Mode								
	123	92%	82%	90%	91%	91%	86%	55%	58%
	143	100%	100%	100%	91%	82%	100%	55%	50%
	151	100%	100%	100%	100%	100%	100%	73%	42%
	163	100%	91%	100%	100%	100%	93%	55%	58%
VII	**High frequency occurrence in Pinelands in winter with intermediate to low frequency in other seasons and si**								
	Positive Mode								
	134	33%	27%	40%	27%	18%	7%	0%	75%
	263	0%	9%	30%	9%	9%	29%	9%	75%
	115	0%	73%	60%	27%	0%	64%	55%	75%
	189	0%	27%	30%	9%	0%	43%	9%	75%
VIII	**Never occur in the spring at either site, but occur at low frequency in other seasons**								
	Negative Mode								
	273	0%	36%	20%	18%	0%	14%	9%	8%
	311	0%	9%	30%	9%	0%	21%	18%	17%
	351	0%	9%	30%	27%	0%	7%	9%	17%
	545	0%	18%	10%	9%	0%	21%	9%	25%
	321	0%	55%	20%	45%	0%	36%	0%	17%
	465	0%	18%	10%	18%	0%	14%	18%	42%

Use of Molecular Marker Database for Chemical Characterization of Rainwater DOM

A second major effort on this project was devoted to the development of a molecular marker database of organic compounds. Our database currently contains over one thousand compounds that have been reported in atmospheric samples in the literature. The database contains information on compound name, structure, CAS number, source, solubility, known sources, functional groups relevant to ESI-MS detection, and literature citation, etc. for many of these compounds. As discussed below, we used this database for targeting compounds to obtain for additional authentic standards, for initial interpretation of compounds in the rain samples from our study sites, and for interpretation of the bioavailability data. This molecular database is key to identifying and quantifying sources of DOM in rainwater. An example of the type of information in the database is shown in Table R5.

An important key in this database is identification of functional groups that determine whether a compound should be detected in the ESI-MS positive or negative ionization mode. As noted above, compounds detected in the positive ESI mode (e.g. polyaromatic hydrocarbons, amines, amides) are expected to have functional groups that are electron donating (or proton accepting) and are thus compounds that are able to “acquire a positive charge”. In ESI-positive compounds with the following basic functional groups should be detected (e.g. -$CH_{3,}$ -NH_2, –OH, -OCH_3, -$N(CH_3)_2$,) (Table 7) (Seitzinger et al., 2003 and 2005). Compounds detected in the negative ESI mode (e.g. amino acids, carboxylic acids, nitrate compounds, carbonitriles) should have electron withdrawing (or proton donating) functional groups and can thus acquire a negative charge. This includes compounds with –NO_2 and COOH functional groups, among others (Table 7).

Potential matches between m/z in the rainwater samples and entries of organic compounds in our Molecular Marker Database are shown in Table 10 for a number of m/z that occur frequently in the Camden and Pinelands samples. These potential matches are based on the rainwater m/z (molecular weight+1 for positive ionization mode; molecular weight-1 for negative ionization mode) and detection in the positive and/or negative ionization mode.

Example 1. One example is m/z 121 which was detected in 100% and 92% of the samples from Camden and Pinelands, respectively. This corresponds to the compound 1,3,5-trimethylbenzene in the Molecular Marker Database. The three methyl groups would suggest that it should be detected in the ESI-positive ionization mode (Table 7). This compound should not be detected in the ESI-negative ionization mode, based on its structure. Examination of the C&P ESI-MS rainwater database showed that there were a few occurrences of m/z 119 (MW-1). However, the ion abundance of m/z 121 in ESI positive mode showed no correlation with the ion abundance of m/z 119 in the ESI-negative mode across the 92 rainwater samples (Figure 4). The lack of correlation between these two ions suggests that m/z 121 detected in positive mode does not have negative mode functional groups. This is consistent with the potential identification of m/z 121 positive as 1,2,5-trimethylbenzene which has been reported from automobile exhaust and biomass combustion (Table 10).

Characterization of the potential number of N atoms in this compound was obtained by applying the “N rule.” If a compound has an odd mass it definitely has a N atom in its structure, and contains an odd number of N atoms (1,3,5 etc.). If a compound has an even mass, it has either no N or an even number of N atoms in its structure (0, 2, 4 etc.). The molecular weight of m/z 121 is projected to be 120 (m/z-1). The N rule therefore predicts that it has either no N or an even number of N atoms. This is consistent, by definition, with the structure of 1,3,5-trimethylbenzene which has no N.

Example 2. A second example is m/z 181 which was detected in 100% of the samples from both the Camden and Pinelands sites. This corresponds to the compound 9H-fluoren-9-one in the

Molecular Marker Database (Table 10). The =O group and its aromatic structure suggest that it should be detected in the ESI-positive ionization mode, but not in the ESI-negative ionization mode (Table 7), which is consistent with the rainwater analyses for that m/z. The sources of 9H-fluoren-9-one are anthropogenic, including automobile exhaust, biomass combustion, coal combustion, diesel, refuse combustion and tobacco smoke (Table 10). The anthropogenic source is consistent with the lack of seasonal pattern in the occurrence of this compound (e.g., Category II compound; Table 9). We have also analyzed a standard of 9H-fluoren-9-one and it is detected in the ESI-positive ion mode, as expected.

Example 3. The Molecular Marker Database also illustrates that more than one compound can have the same unit mass resolution and expected positive/negative mode detection. For example there are three entries for expected positive mode detection at an m/z of 123 (Table 10). The C&P ESI-MS rainwater database showed that there was a high frequency of occurrence of m/z 123 in the positive mode.

Additional analysis is required to narrow down the chemical identity of these and other masses in the rainwater samples.

Table 10. Examples of entries in Molecular Marker Database of atmospheric organic compounds based on information previously reported in the literature. Also indicated are examples of potential matches with m/z detected in Camden and Pinelands rainwater.

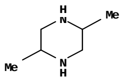
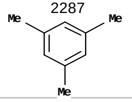
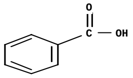
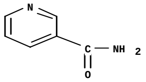
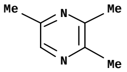
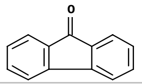
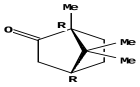
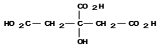
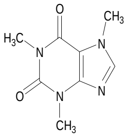
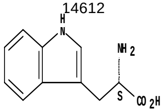
Registry #	CAS Name / Species	CRC No. &/or Structure	Group ID	Formula	MW	Solubility	Dissociation Constants	Reference	Source	Functional Group (pos)	Functional Group (neg)	Percent Occurrence of m/z in Camden rainwater	Percent Occurrence of m/z in Pinelands rainwater
106-55-8	2,5-dimethyl-piperazine		heterocyclic Nitrogen	C6H14N2	114.19	Molar solubility very soluble@pH 1,4,7,8,10	Step 1 9.66 / 25 Step 2 5.20 / 25; pKa=9.99±0.20	Graedel	G-diesel	2CH3, cyclic, N-heterocycle			
108-67-8	1,3,5-trimethylbenzene	2287 	monocyclic arene	1,3,5-(CH3)3-C6H3; C9 H12	120.20	Very soluble in al, eth, ace, bz		Graedel; Holes et al., 1997	auto; biomass combustion	3CH3, aromatic		100% (+ mode; m/z 121)	92% (+ mode; m/z 121)
65-85-0	benzoic acid		aromatic acid	C6H5CO2H	122.13	al, eth, ace, bz, chloroform (chl)	pKa 4.20 0.20 Most Acidic	Holes et al., 1997; Graedel	animal waste; auto	aromatic, OH	COOH	93% (+ mode; m/z 123)	92% (+ mode; m/z 123)
98-92-0	3-pyridinecarboxamide		heterocyclic nitrogen	3-C5H4N CONH2; C6 H6 N2 O	122.13	Very soluble @ pH 1,4; Soluble @ pH 7,8,10	pKa 3.54 0.11 Most Basic	Saint-Jalm & Moree-Testa, 1980; Graedel	tobacco smoke	aromatic, N-heteroatom, NH2, =O		93% (+ mode; m/z 123)	92% (+ mode; m/z 123)
14667-55-1	trimethylpyrazine		heterocyclic nitrogen	(CH3)3C4HN2 ; C7 H10 N2	122.17	pH 1; Very Solubl	pKa 2.43 0.20 Most Basic	Graedel	rendering; sewage treatment	3CH3, aromatic, N-heteroatom		93% (+ mode; m/z 123)	92% (+ mode; m/z 123)
486-25-9	9H-fluoren-9-one		aromatic hydrocarbon	C13H8O	180.20	Sparingly Sol @ ph 1,4,7,8,10		Graedel	auto; biomass comb.; coal comb.; diesel; refuse comb.; tobacco smoke;	=O		100% (+ mode; m/z 181)	100% (+ mode; m/z 181)
124-18-5	decane	$Me-(CH_2)_8-Me$	alkanes	C10H22	142.29	Sparingly Sol. @ pH 1,4,7,8,10		Luxenhofer et al., 1996; Graedel	adhesives, auto	2CH3		97% (+ mode; m/z 143)	81% (+ mode; m/z 143)
464-49-3	1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one		cyclic ketones	C10H16O	152.24	Sparingly Sol @ ph 1,4,7,8,10		Graedel; Rogge et al., 1993	chemical manufacture, vegetation	3CH3, =O		100% (+ mode; m/z 153)	100% (+ mode; m/z 153)

Table. 10 (continued)

Registry #	CAS Name / Species	CRC No. &/or Structure	Group ID	Formula	MW	Solubility	Dissociation Constants	Reference	Source	Functional Group (pos)	Functional Group (neg)	Percent Occurrence of m/z in Camden rainwater	Percent Occurrence of m/z in Pinelands rainwater
26844-80-4	5-methyl-[2,3']bipyridinyl		heterocyclic nitrogen	C11H10N2	170.21			Graedel	G-tobacco smoke			100% (+ mode; m/z 171)	100% (+ mode; m/z 171)
77-92-9	2-hydroxy-1,2,3-propanetri carboxylic acid	 $HO_2C-CH_2-\underset{OH}{\overset{CO_2H}{C}}-CH_2-CO_2H$	alkanic acid	HOC(CH2CO 2H)2CO2H	192.14	W, al, eth	pKa 2.93±0.28 Most Acidic	Graedel; Saxena & Hildemann, 1996		4OH	3COOH	97% (+ mode; m/z 193)	96% (+ mode; m/z 193)
58-08-2	3,7-dihydro-1,3,7-trimethyl-1 H-purine-2,6-dione		heterocyclic nitrogen	C8H10N4O2	194.19	One gram dissolves in 46 ml water, 5.5 ml water at 80degrees, 1.5 ml boiling water; Soluble @ pH 1,4,7,8,10	pKa 1.39±0.70 Most Basic	Graedel	refuse comb.	2=O, 3CH3, N-heteroatoms		93% (+ mode; m/z 195)	96% (+ mode; m/z 195)
73-22-3	tryptophan	14612 	amino acid	C11H12N2O2	204.23	Very Sol @ pH 1,4,7,8,10	pKa 2.31±0.10 Most Acidic pKa 9.51±0.16 Most Basic	Mopper & Zika, 1987	biogenic	aromatic, OH, NH2, N-heteroatom	COOH	100% (+ mode; m/z 205)	88% (+ mode; m/z 205)

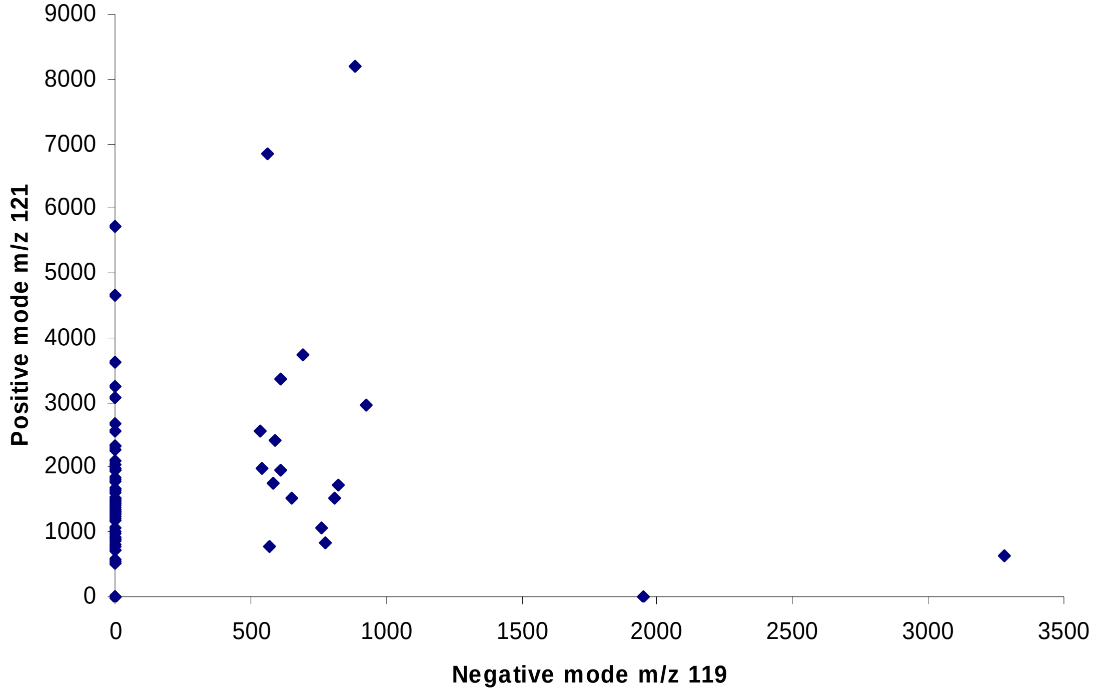


Figure 4. Ion abundance of m/z 121 in ESI positive mode compared to m/z 119 in ESI negative mode for all 92 rainwater samples. The lack of correlation between these two ions suggests that m/z 121 detected in positive mode does not have negative mode functional groups. This is consistent with the potential identification of m/z 121 positive as 1,2,5-trimethylbenzene in our Molecular Marker Database of Atmospheric Organic Compounds (Table 10).

In the above sections we outlined a number of specific approaches that we developed to begin to identify specific compounds and their sources in the rainwater at the Camden and Pinelands sites. This included:

1) determining the m/z and ion abundance of each compound (with subsequent entry into our C&P ESI-MS rainwater database)
2) filtering the database for m/z with very low frequency of occurrence
3) identifying potential functional groups of each m/z based on ESI-negative and/or positive mode detection and knowledge gained from analysis of standard compounds with known functional groups

4) identifying which functional groups are not likely to occur in the compound based on whether a companion m/z is detected in the opposite ESI-ionization mode.

5) evaluating the number of N atoms in a compound by applying the "N rule"

6) making potential matches between rainwater m/z and entries in our Molecular Marker Database based on m/z and positive/negative mode detection

7) identifying groups of m/z's that show similar patterns of occurrence and concentration (ion abundance) with season and site and which thus indicate similar sources or source types

Using this information we then targeted specific compounds in the rainwater samples for further chemical characterization. This included determining response factors for standards and those compounds across a range of instrument operating conditions and determining their retention characteristics on LC columns.

Further Identification of Specific Organic Compounds

Selected masses from the rainwater samples and their potential matches with compounds in the Molecular Marker Database of Atmospheric Organic Compounds were analyzed across a range of instrument operating conditions. The most useful was found to be varying fragmentor voltage. The results of these tests are summarized below.

1) The ESI-MS response to authentic standards across a range of fragmentor voltages (FV) is repeatable from day to day (Appendix Figure. A-1). Different compounds show different patterns of response. Results for 4 standard compounds analyzed on two separate days demonstrate the repeatability of results.

2) Samples spiked with standards show the same or similar ESI-MS response across a range of FV indicating that there are minimal matrix effects from the rain water (Appendix Figure. A-2). Four samples (2 Camden and 2 Pinelands) were spiked with each of the four standard compounds and compared to the ESI-MS response to the standards in deionized water. There were some notable exceptions to the responses. In particular, the response of the spiked samples to 2,5,-dimethylpiperazine, phthalic acid, and 1,6-heaxanediamine at a FV of 60 varied from the pure standards. Also, at 120 FV the phthalic acid spiked samples always showed an increase in response. As noted below, these are important additional diagnostics and demonstrate that sample spikes are important for compound identification.

3) We made significant additional progress in beginning to identify compounds in the rainwater samples using the above information. Specifically, we compared the ESI-MS response across a range of FV to an m/z in (unspiked) rainwater samples, to the same m/z in samples spiked with authentic standards, and to standards in deionized water. (Appendix Figs. A-3 to A-6). The results strongly suggest that all four of the compounds examined, occur in one or more of the four Pinelands and Camden rainwater samples.

Phthalic acid: For example, all phthalic acid spiked samples showed a decrease in response to m/z 165 (negative ionization mode) between 40 and 80 volts and then a marked increase in response at FV 120 (Appendix Figure. A-3). The m/z 165 in all four unspiked rain samples exhibited the same overall pattern, as well as the response at FV 120. This strongly indicates that m/z 165 is phthalic acid in all four samples. Furthermore, we can now calculate the expected concentration of phthalic acid in these samples based on the response factor calculated from the 0.5 µM phthalic acid sample spikes and the ion abundance for m/z 165 in the (unspiked) rainwater samples. The predicted concentration of phthalic acid is ranges from 0.33 to 0.54 µM (Table 11).

1,6-Hexanediamine: Camden sample CR10811 showed a very similar ESI-MS response to m/z 117 (positive ion mode) in the unspiked and spiked sample across the range of FV (Appendix Figure. A-4). The response in the other three samples was similar except for the slight increase in ion abundance at FV 100 and 120 in the spiked but not un-spiked sample. Further analyses are required including HPLC analyses to further confirm the identity. We made a preliminary calculation of the concentration of 1,6-Hexanediamine for CR10811 (Table 11).

Citraconic acid: The ESI-MS response to m/z 129 in the three rainwater samples was similar to the citraconic acid standard (Appendix Figure. A-5). This indicates that m/z 129 in these samples is citraconic acid. The predicted concentration of citraconic acid was approximately 0.2 µM in the two Camden and one Pinelands samples (Table 11). No citraconic acid was indicated to occur in the 14 August 2001 Pinelands sample.

2,5-Dimethylpiperazine: This compound does not appear to occur in three of the four samples based on the very different response of the ESI-MS in the spiked and unspiked samples for CR010811, PR10727 and PR010814 (Appendix Figure. A-6). It is not clear if Camden sample CR010814 contains 2,5-Dimethylpiperazine. Further analyses are required.

The above examples illustrate the use of this approach toward positive identification of a range of compounds in the Pinelands and Camden rain. It also illustrates how we can begin to link masses to specific source-types. The next step was to confirm compound identity using HPLC.

Table 11. Estimated concentration of compounds in rain samples from Camden and Pinelands based on ESI-MS response across a range of FV to the same m/z in samples not spiked and spiked with authentic standards.

Sample	**Phthalic acid m/z 165 negative**	**1,6-Hexanediamine m/z 117 positive**	**Citraconic acid m/z 129 negative**
Camden CR10811	0.33 µM	3.5 µM	0.2 µM
Camden CR10814	0.54 µM	?	0.2 µM
Pinelands PR10814	0.39 µM	?	0
Pinelands PR10727	0.39 µM	?	0.15 µM

Table 12. Standard compounds analyzed under a range of ESI-MS fragmentor voltages (FV) and/or using a C18 column (HPLC) for compound identification.

Compounds	**MW**	**Mode**	**FV**	**HPLC**
Urea	60	Pos	√	√
Imidazole	68	Pos	√	√
Allylurea	100	Pos	√	√
Caffeine	194	Pos	√	√
Benzaldehyde	106	Pos	√	
Camphor	152	Pos	√	
Naphthalene	128	Pos	√	
Benzoic Acid	122	Pos/Neg	√	√
1,6-Hexanediamine	116	Pos	√	
1-Nitronaphthalene	173	Pos	√	
2,6-Dimethylpiperazine	114	Pos	√	
3-Phenylpropionic acid	150	Neg	√	
Acrylic acid	72	Neg	√	
Citraconic acid	130	Neg	√	
Histidine	155	Neg	√	
Nonanoic acid	158	Neg	√	
Malonic acid	104	Neg	√	
Maleic acid	116	Neg	√	
Phthalic acid	166	Neg	√	
PAH (16)	several	Pos	√	
KNO3-	62	Neg		√
Succinic acid	118	Neg		√
Glutaric acid	132	Neg		√
Malic acid	134	Neg		√
Adipic acid	146	Neg		√
Nitrobenzoic acid	167	Neg		√
Azelaic acid	188	Neg		√
DL-Lactic acid	90	Pos/Neg		√
Oxalic acid	90	Pos/Neg		√
Glycolic acid	76	Pos/Neg		√
Citric acid	192	Pos/Neg		√
Amino Acid Mix (20)	several	Pos/Neg		√
Propionic acid	74	Pos/Neg		√
Acetic acid	60	Pos/Neg		√
L-Ascorbic acid	176	Pos/Neg		√
Glucosamine Hydrochloride	216	Pos/Neg		√
L-Leucine	131	Pos/Neg		√
Hexamethylenetetramine	140	Pos/Neg		√

HPLC Analysis Combined with ESI-MS

Further identification of compounds that potentially represent specific source types was conducted using HPLC in combination with ESI-MS. Masses were selected for this analysis based on the following criteria: high frequency of occurrence in rainwater samples, matches with compounds in the Molecular Marker Database of Atmospheric Organic Compounds based on molecular weight and positive/negative mode detection, compounds representing different source types (natural, various anthropogenic sources). Examples of compounds chosen were L-leucine, glucosaminehydrochloride, succinic acid, 1,6-hexamethelyenediamine, and benzoic acid.

DOM compounds in selected rainwater samples (2 Camden; 3 Pinelands) were separated using HPLC (C18 column) in tandem with ESI-MS. Standards of the above compounds (in DIW water) were analyzed under the same operating conditions and the retention times determined (Table 12). We analyzed both single compound standards and mixed compound standards. The retention time of the specific standard compound was then compared to the retention time (RT) of the target m/z in the samples. Samples were then spiked with the standard and analyzed, and again the retention time of the spike was compared to the retention time of the target m/z in the samples. In numerous cases the RT of the target mass in the sample and the standard were the same. For example, the retention time of the L-leucine standard in DIW and spiked into the rainwater samples was the same as the target mass in the sample (1.8 minutes). This strongly indicates that L-leucine is mass 132 (positive mode detection) in these rainwater samples. However, the use of the LC column introduced considerable noise into the ESI-MS results which in numerous samples and for various standards made it very difficult to determine exact retention times, due to the low concentration of any one compound in the rainwater. To overcome this problem, we tried concentrating several rainwater samples (five fold concentration) and repeating the analysis. While this improved the signal to noise ratio, considerable further work would be required to perfect this approach.

Next Steps in Identification of Specific Compounds

We advise that the next step in obtaining positive chemical identification of the large suite of individual organic compounds in rainwater, and therefore to further the identification of source-types, is to continue to use ESI-MS, but to move to the use of ultrahigh resolution ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR MS). The suggested use of FT-ICR is based on work conducted outside of the present NJDEP project in which we analyzed DOM from a number of aquatic samples using FT-ICR MS at the National High Magnetic Analytical Facility in Florida. ESI FT-ICR MS provides the same type of information as the ESI-MS used in the current project. However, the ultrahigh resolution capabilities of the 9.4 Torr instrument provides five decimal place resolution of the mass of each compound (compared to unit mass with our ESI-MS). Therefore, *absolute elemental composition of each compound can be obtained.* That information would then be interpreted in conjunction with our Molecular Marker Database of Atmospheric Organic Compounds to make additional major advances in identification of source-types of each compound. The current project lays the foundation for that work. We suggest FT-ICR MS analysis of approximately 10 -15 rainwater samples selected from our suite of preserved rainwater samples which were collected during the present project. Those samples would be chosen based on the presence of frequently occurring masses in a wide number of samples, and masses with specific site, season and storm trajectory occurrence.

DOM Composition of Rainwater Collected from Other Storm Trajectories

Almost all samples collected during the 24-month sampling period were from storms with a southern and western trajectory, which is the predominant storm trajectory in this region. During Year 3, we specifically targeted our collections to rainwater from eastern (including southeastern) and northern trajectories. Four storms, with collections from both Camden and Pinelands, were sampled (8 samples) (Table 6). Three of those storms had an easterly component to them, and one a NW component. The easterly samples could potentially include more of a marine source and less of a terrestrial source (natural or anthropogenic). All samples were analyzed for bulk constituents and by ESI-MS.

Overall bulk nutrient concentrations and pH were within the range of values from samples collected from the southern and western trajectories during the 24-month sampling period (Fig. 1 and Table 6).

Detailed comparison of the specific trajectory samples with the previous (24-month) samples includes a number of uncertainties for the following reasons. There is a very uneven sample size; there were only 8 samples (5 storms) compared to 92 samples. The four storms were spread over three seasons (fall, winter and spring), hence there was basically only one storm per season. The samples had a generally low DOC concentration which is consistent with previous reports of rainwater collected from marine locations (Cornell et al. 1995). We had major instrument problems and thus extensive repair of our ESI-MS between the time that analysis of the 24-month samples was completed and the specific trajectory samples were analyzed. While the ESI-MS was operating very well during the analysis of all samples, and results of standards analyzed with each run were similar, small differences in instrument sensitivity due to changes in internal calibration and changes in electronics can complicate detailed comparison of samples analyzed before and after repair. With these caveats in mind, the following observations were made.

The overall molecular weight distribution of compounds in these four storms was similar to previous samples from a generally SW, WSW direction. However, there were a number of differences in patterns of occurrence of specific masses. Numerous masses which occurred at high frequency in storms from the SW/WSW direction did not occur in any (or only 1) of the eight samples from the easterly or NW direction (Table 13). A number of additional masses occurred with high frequency in the storms from the easterly or NW direction but had low occurrence in storms form the SW/WSW. While a number of caveats must be considered in comparing these two sets of samples, as noted above, the results suggest that storms with different trajectories have notable differences in organic matter chemical composition, indicative of some differences in sources.

Table 13. Examples of masses showing different patterns of occurrence between storms from a generally SW/WSW trajectory (92 samples) relative to storms from a SE or NW trajectory (8 samples). See text for details.

	Generally SW & WSW		**SE or, NE or NW Storms**			
MZ	**Camden**	**Pinelands**	**Camden**	**Pinelands**	**Camden**	**Pinelands**
	% Occurrence		**# of occurrences: max 4**		**% Occurrence**	
<u>No occurrences in SE or NW trajectory storms but high frequency in SW and WSW storms</u>						
301	80%	79%	0	0	0%	0%
303	73%	52%	0	0	0%	0%
131	70%	40%	0	0	0%	0%
156	70%	33%	0	0	0%	0%
162	70%	56%	0	0	0%	0%
268	70%	44%	0	0	0%	0%
120	68%	50%	0	0	0%	0%
163	98%	69%	0	0	0%	0%
111	91%	73%	0	0	0%	0%
219	80%	75%	0	0	0%	0%
243	80%	56%	0	0	0%	0%
277	80%	67%	0	0	0%	0%
279	77%	69%	0	0	0%	0%
293	86%	46%	0	0	0%	0%
<u>Low frequency in SE or NW trajectory storms but high frequency in SW or WSW storms</u>						
129	77%	42%	0	1	0%	25%
285	77%	54%	0	1	0%	25%
144	68%	29%	1	0	25%	0%
186	70%	48%	1	0	25%	0%
121	91%	67%	1	0	25%	0%
245	91%	60%	1	0	25%	0%
291	84%	63%	1	0	25%	0%
175	82%	58%	2	0	50%	0%
<u>High frequency in SE or NW trajectory storms but low frequency in SW or WSW storms</u>						
91	32%	23%	4	3	100%	75%
83	11%	6%	4	2	100%	50%
74	0%	4%	2	3	50%	75%
81	55%	46%	4	3	100%	75%
102	18%	6%	2	2	50%	50%

Bioavailability of DOM in Rainwater

The third major question addressed in this project was:

Which of the DOM compounds, and therefore which potential sources, are bioavailable and thus contributing to ecological changes in ecosystems?

At the same time that we developed and applied the above ESI-MS based analytical approach to chemically characterize the DOM compounds in rainwater, we also developed new approaches to determine which of these compounds are biologically used once they are deposited to ecosystems. We know from the few studies conducted to date, that some portion, although not all, of the bulk (total) DOM in rainwater is biologically available (Peierls and Paerl 1997; Seitzinger and Sanders 1999). For example, between 40% and 70% of the bulk DON in rainwater collected from the Philadelphia, PA, area was utilized by coastal plankton (Seitzinger and Sanders 1999). Experiments conducted with rainwater from New Brunswick, NJ, indicated that approximately 5% to 50% of the total DON was utilized by aquatic microorganisms, with different amounts of DON utilized in different rainfall events (Seitzinger, et al. in prep.). However, none of these studies identified the chemical characteristics of bioavailable or refractory DOM compounds or their sources. Therefore, a major advance in the current project was to determine which components (compounds) of the DOM in NJ rain are utilized by organisms and thus causing changes to receiving ecosystems, and to provide insight into what the sources of those compounds are.

In the current project we examined the utilization of the DOM compounds in rainwater collected from the urban Camden site and from the relatively undisturbed Pinelands site. The basic approach was to analyze bulk DOM and the detailed molecular level chemical composition of the rain water before and after degradation by aquatic bacteria. A comparison of the chemical characteristics of the utilized (and un-utilized) compounds was conducted. The ESI-MS results for individual m/z were then compared to compounds in our Molecular Marker Database of Atmospheric Organic Compounds and with the response of authentic standards of atmospheric relevance to begin to provide information on which specific types of processes (e.g., combustion, manufacturing processes, vehicular exhaust, photochemical, natural, etc.) are contributing biologically available DOM. Such information is necessary to begin to develop models to predict changes in ecosystems due to changes in human activities that contribute organic-N and C to the atmosphere. It will also provide information that could be used to determine what sources would be the most effective to control in order to decrease effects on receiving ecosystems.

Changes in Bulk DOM and in Bacterial Production

An equivalent amount of DOC was utilized in both Camden & Pinelands treatments (92 and 98 µM-C, respectively) over the two week incubation period (Figure 5). DON concentrations in both treatments also decreased significantly; a net total of 7.2 µM-N and 5 µM-N were taken up in the Camden and the Pinelands rainwater, respectively (Figure 6).

The bacterial community in both treatments used bulk DOM in the same C:N ratio as that measured at the beginning of the incubations, although the DOC:DON ratio varied throughout the incubations. The DOC:DON ratio in Camden and Pinelands samples and bio-available fractions was ~13 and ~20, respectively.

Bacterial growth rates showed a similar trend in the rainwater incubations from both sites; microbial activity peaked on Day 3 in both rainwater treatments, and decreased thereafter with increasing time (Figure 5). Despite this and the similar amounts of DOM utilized, bacterial production was three times higher in the Pinelands (10.6±0.8 µg-C.L^{-1}.hr^{-1}) compared to the Camden treatment (4.0±1.0 µg-

C.L^{-1}.hr^{-1}) (Figure 6). This indicates that in the Pinelands rainwater, a larger proportion of the DOM was utilized towards bacterial growth than in the Camden rainwater.

The bioavailable pool of DOM in both rainwater treatments was utilized rapidly within the first week of the experiment (Figure 5). Approximately 50% of the rainwater DOM was thus very labile (i.e., consumed in the order of hours to days). The remainder (Pinelands 45%, Camden 55%) of the DOM pool comprised semi-labile to refractory compounds that were unavailable to the microbial community.

Of the total DOC and DON utilized by the bacteria during the course of the experiment, ~55 µM-DOC and ~11.0µM-DON in Camden rainwater and ~161µM-C and 32.2µM-N of the Pinelands DOM pool was used for growth alone (assuming the C:N ratio of bacterial biomass to be 5). It is well known that there are inherent uncertainties with converting bacterial production rates to C and N uptake by using leucine and thymidine incorporation assays. However, both Camden and Pinelands estimates of DOM used for growth are in the same order of magnitude as the observed net utilization of DOM.

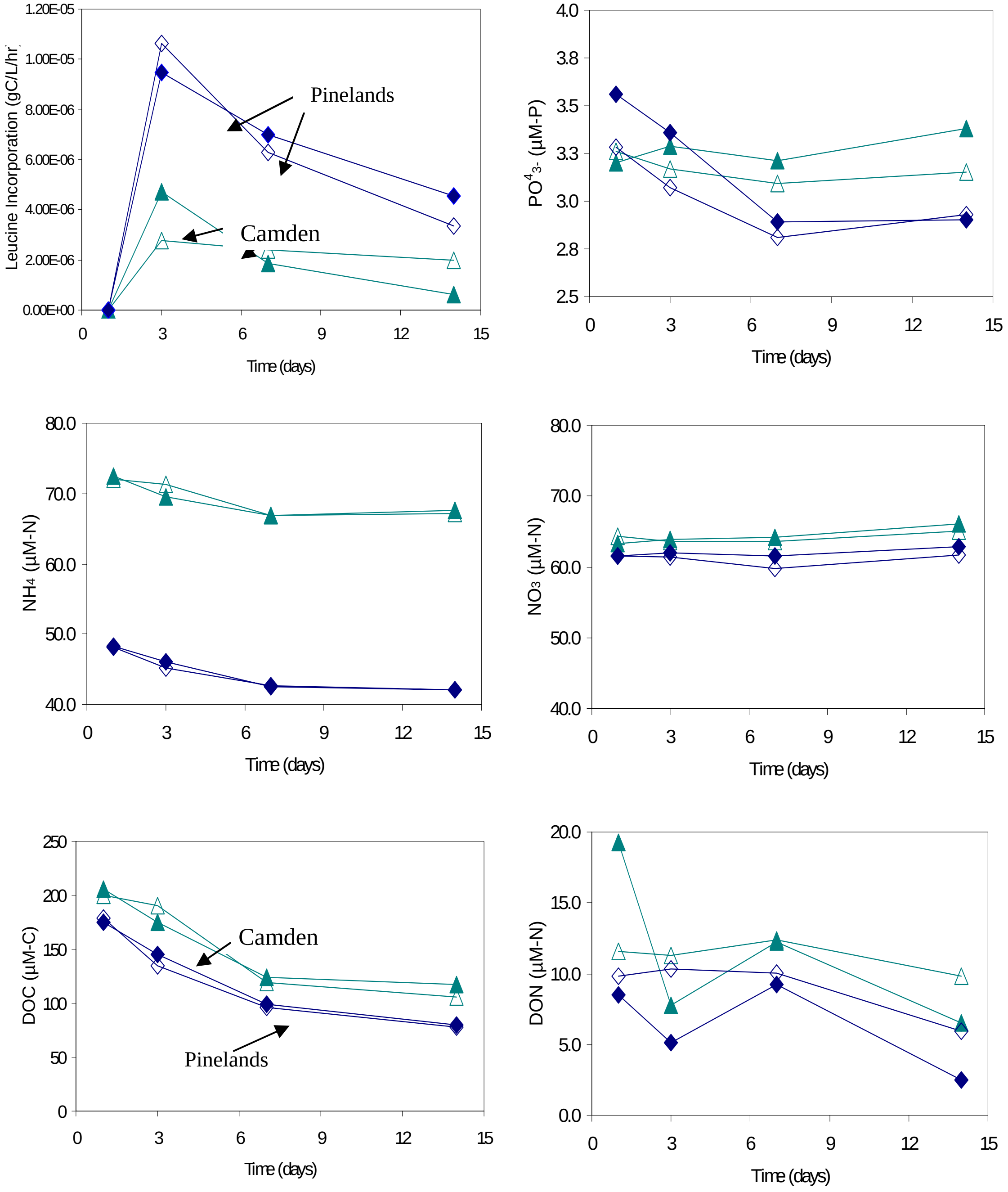


Figure 5 Bulk chemical components (PO_4^{3-}, NO_3^-, NH_4^+, DON, DOC) and bacterial production in the Camden and Pinelands rainwater treatments incubated for 14 days (Camden Replicate A – open triangles, Camden Replicate B – closed triangles; Pinelands Replicate A – open diamonds, Pinelands Replicate B – closed diamonds).

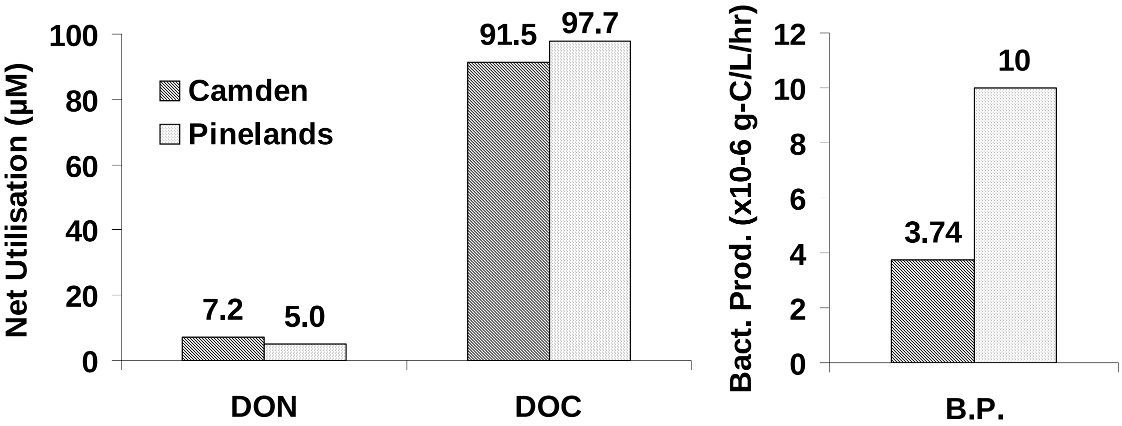


Figure 6. Net utilization of DOM in Camden and Pinelands rainwater inoculated with bacteria (right panel); Maximum bacterial growth rates (max bacterial production; BP) in Camden and Pinelands rainwater incubations (left panel).

Chemical Characterization of Bioavailable and Refractory DOM in Rainwater

The bulk DOM pool is thought to be comprised of compounds of varying biological availability. Typically, it is separated into labile, semi-labile and refractory pools based on bulk DOM measurements from degradation experiments similar to this one (Seitzinger et al. 2002; recently reviewed by Bronk 2002 and del Giorgio and Davis 2003). However, previously it has not been possible to examine the uptake of the whole suite of organic compounds or m/z's at the molecular level, except with the application of compound-specific analyses which track only groups of compounds such as total hydrolysable amino acids, sugars and fatty acids. Our ESI-MS results provide a more in-depth look at bacterial utilization of organic compounds as we can track the change in concentration of each m/z through time based on its ion abundance. Compounds or m/z's were grouped in the general categories of "bioavailable", "refractory" and "new/increased" depending on whether they: a) decreased in ion abundance partially or totally, b) did not significantly change in ion abundance, or c) appeared or increased in ion abundance by the end of the experiment, respectively. We note that while ESI ion abundance is a measure of compound concentration, the relationship between ion abundance and concentration is compound specific because of differences in ionization efficiencies among compounds. A number of factors affect ionization efficiencies including compound structure, mobile phase conditions, and the sample matrix. However, because the response of the ESI-MS to compounds is generally linear over a range of concentrations (nM to tens of µM), we can tell approximately what proportion of the total concentration of an m/z was utilized (e.g., a 40% decrease in ion abundance for a particular m/z is approximately equivalent to a 40% decrease in concentration of that m/z) by following the change in ion abundance for a specific m/z through time in samples from the bioavailability experiment.

Approximately 50% of the m/z's in both rainwater samples were utilized wholly or partially by the microbial community (Figure 7). This included compounds that exhibited either basic and/or acidic functional groups. Although a similar percent of m/z were labile between the two sites, including compounds with positive and\or negative functional groups, this is not mirrored in the refractory and new/increased fractions of rainwater DOM. Approximately ~30% of compounds with basic functional groups and ~15% of compounds with acidic functional groups remained unchanged. Furthermore, a significant number of compounds with acidic groups were produced by the bacteria.

The overall changes in ion abundance suggest that there was a smaller amount of refractory compounds and a slightly larger amount of bioavailable compounds in the Pinelands rainwater (Figure 8). This fits with the bacterial production data that suggested higher bacterial production in the Pinelands compared to Camden rainwater. More detailed molecular level information is required to link differences in bacteria production to compound specific differences in bioavailable compounds between the two sites.

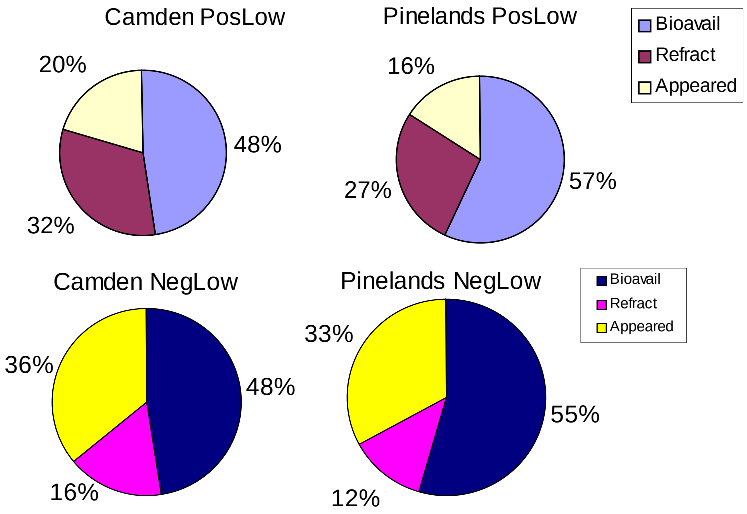


Figure 7. Number of m/z peaks that were either taken up by the bacterial community (bioavailable), did not change concentration over time (refractory), or were produced (new/increased).

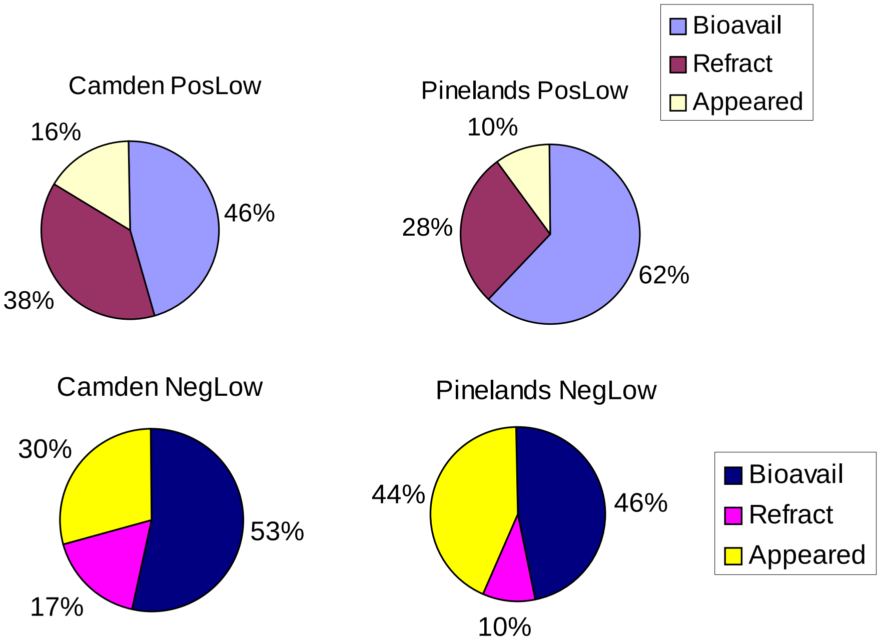


Figure 8. Total ion abundance that was either consumed by the bacterial community (bioavailable), did not change concentrations over time (refractory), or were produced (new).

Use of Molecular Marker Database for Chemical Characterization of Bioavailable DOM Compounds in Rainwater

Integration of the ESI-MS data with our Molecular Marker Database of Atmospheric Organic Compounds was then used to begin to link specific types of human activities/processes to bioavailable chemical compounds in the rainwater. We were able to make some preliminary matches between the m/z peaks in the rainwater that were bioavailable and compounds in our Molecular Marker Database of Atmospheric Organic Compounds. Over 25 bioavailable m/z's in the rainwater compounds have matches in the Molecular Marker Database (Table 14). We focused this effort on m/z that were bioavailable in both the Camden and Pinelands rainwater. These matches were based on molecular weight (m/z+1 for negative mode detected compounds and m/z-1 for positive mode detected compounds) and expected (Molecular Marker Database) or measured (rainwater samples) positive and/or negative ionization mode detection. These potential matches include, among others, compounds from anthropogenic sources such as styrene from adhesives and automobile exhaust, 2-methyl benzonitrile from polymer combustion, methenamine from explosive manufacture, and fluoranthene from aluminum manufacture and asphalt paving. Also included are matches with compounds of natural, biogenic origin such as tocopherol acetate from vegetables. It must be emphasized that these are potential matches based on the criteria noted above.

Further analyses are required to obtain absolute chemical identity of the bioavailable and refractory compounds in the rainwater, and to determine the amount used of specific compounds and compound groups. Such information could then more specifically link source categories/human activities to potential ecological changes. As detailed in previous sections, a number of approaches were explored. The next step suggested is the use of ultrahigh resolution mass spectrometry, specifically ESI-FT-ICR MS.

Table 14. Examples of compounds (m/z) that were bioavailable and their potential matches with compounds in our Molecular Marker Database of Atmospheric Organic Compounds. The potential matches were based on m/z and measured (rainwater) or predicted (Molecular Marker Database) in positive and/or negative ESI ionization mode.

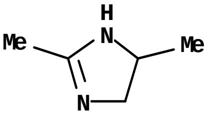
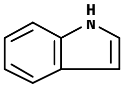
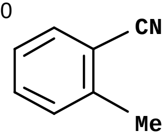
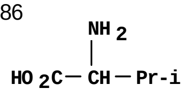
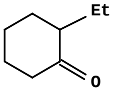
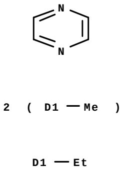
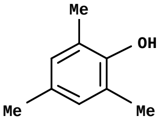
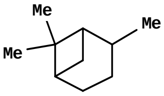
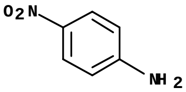
Registry #	Compound	Structure	Group ID	Formula	MW	Source
930-61-0	98.15	4,5-dihydro-2,4-dimethyl-imidazole		Heterocyclic Nitrogen	2,4-(CH3)2-C3H2N2	turbine
56-12-2	103.12	4-aminobutanoic acid	$H_2N-(CH_2)_3-CO_2H$	amine	C4H9NO2	volcano
100-42-5	104.16	ethenyl-benzene; styrene	$H_2C{=}CH-Ph$	monocyclic arene	C6H5CH=CH2; C8H8	adhesives, auto
120-72-9	117.15	1H-indole		Heterocyclic Nitrogen	C8H7N	animal waste, coal comb.; tobacco smoke
529-19-1	117.15	2-methyl benzonitrile	14270 	nitriles	2-CH3C6H4N; C8 H7 N	polymer combustion
640-68-6	117.15	valine (D)	14886  $HO_2C-\overset{NH_2}{CH}-Pr\text{-}i$	amino acid	(CH3)2CH CH(NH2)CO2H	
4423-94-3	126.2	2-ethylcyclohexanone		cyclic	2-C2H5-C6H9OH; C8 H14 O	
71607-73-3	136.2	ethyldimethylpyrazine		Heterocyclic Nitrogen	C2H5(CH3)2-C4HN2; C8 H12 N2	rendering; sewage treatment
527-60-6	136.2	2,4,6-trimethyl-phenol		aromatic alcohol	2,4,6-(CH3)3C6H2OH; C9 H12 O	auto; diesel; tobacco smoke
1330-16-1	136.24	2,6,6-trimethyl-bicyclo[3.1.1]heptane; pinene		terpenes	C10H16	
100-01-6	138.13	4-nitroaniline; benzenamine,	955 	Aromatic Nitro	4-NO2-C6H4NH2	industrial

Table 14 – (continued)

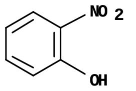
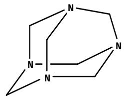
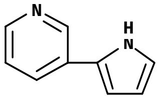
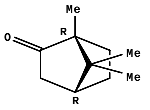
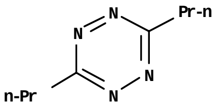
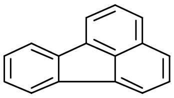
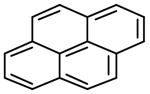
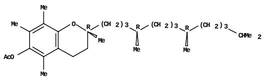
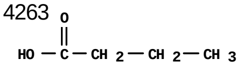
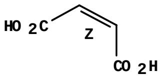
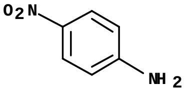
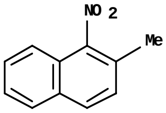
Registry #	Compound	Structure	Group ID	Formula	MW	Source
88-75-5	139.11	2-nitrophenol		Aromatic Nitro	NO2C6H4 OH; C6 H5 N O3	
100-02-7	139.11	4-nitrophenol		Aromatic Nitro	NO2C6H4 OH; C6 H5 N O3	
100-97-0	140.19	1,3,5,7-tetraazatricyclo[3.3.1.13,7]-decane; methenamine		Heterocyclic Nitrogen	C6H12N4	explosive mfr.
494-98-4	144.18	3-pyrrol-2-yl-pyridine; nornicotyrine		Heterocyclic Nitrogen	C9H8N2	G-tobacco smoke
464-49-3	152.24	1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one; camphor		cyclic ketones	C10H16O	chemical manufacture, vegetation
13717-92-5	166.23	3, 6-dipropyl-1, 2, 4, 5-tetrazine		Heterocyclic Nitrogen	3,6-(C3H7)2-C2N4; C8 H14 N4	turbine
	182.17	methylbenzene-1,4-dicarboxylic acid		cyclic	C6H5CH3-1,4-(CO2H)2	
206-44-0	202.26	fluoranthene		higher polycyclic arenes	C16H10	aluminum manufacture; asphalt paving
129-00-0	202.26	pyrene		higher polycyclic arenes	C16H10	aluminum manufacture; asphalt paving
58-95-7	472.75	α-tocopherol acetate (6CI)			C31H52O3	vegetables; found in tomato juice & carrot juice
107-92-6	88.12	butanoic acid; butyric acid	4263  $HO-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-CH_3$	alkanic acid	CH3CH2C H2CO2H	animal waste; chemical manufacture
141-82-2	104.06	propanedioic acid; malonic acid	$HO_2C-CH_2-CO_2H$	alkanic acid	HO2CCH2 CO2H; C3 H4 O4	tobacco smoke
110-16-7	116.07	2-butenedioic acid; maleic acid		olefinic acid	HO2CCH= CHCO2H	
142-62-1	116.16	hexanoic acid; caproic acid	$Me-(CH_2)_4-CO_2H$		CH3(CH2)4 CO2H	

Table 14 (continued)

Registry #	Compound	Structure	Group ID	Formula	MW	Source
110-15-6	118.09	butanedioic acid; succinic acid	13536 $HO_2C-CH_2-CH_2-CO_2H$	alkanic acid	HO2CCH2 CH2CO2H	biomass combustion; tobacco smoke
100-01-6	138.13	4-nitroaniline; benzenamine,	955 	Aromatic Nitro	4-NO2- C6H4NH2	industrial
881-03-8	187.2	2-methyl-1-nitronaphthalene	9401 	Aromatic Nitro	2-CH3-1-NO2-C10H6; C11 H9 N O2	diesel

References

Benner, R. and M. Strom. 1993. A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. Marine Chemistry 41:153-160.

Blando, J.D. and B.J. Turpin. 2000. Secondary organic aerosol formation in cloud and fog droplets: a literature evaluation of plausibility. Atmos. Environ. 34:1623-1632.

Bronk, D.A. 2002. Dynamics of DON, p. 153-247. *In* D.A. Hansell and C.A. Carlson [eds.], Biogeochemistry of Marine Dissolved Organic Matter. Elsevier.

Cornell, S., A. Rendell, and T. Jickells. 1995. Atmospheric inputs of dissolved organic nitrogen to the oceans. Nature 376: 243-246.

del Giorgio, P.A. and J. Davis 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399-424. *In* S. Findlay and R. Sinsabaugh [eds.], Aquatic Ecosystems. Elsevier.

Finlayson-Pitts, B. and J. N. Pitts Jr. 1986. Atmospheric Chemistry: Fundamentals and Experimental Techniques, John Wiley and Sons, New York, NY.

Finlayson-Pitts, B.J. and J.N. Pitts, Jr. 1997. Tropospheric air pollution: Ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles. Science, 276(5315): 1045-1052.

Fisher, D., J. Ceraso, T. Mathew and M. Oppenheimer. 1988. Polluted coastal waters: The role of acid rain. New York, Environmental Defense Fund.

Fonselius, S. 1954. Amino acids in rainwater. Tellus, 6, 90.

Galloway, J.N., W.H. Schlesinger, H. Levy II, A. Michaels, and J.L. Schnoor. 1995. Nitrogen fixation: Anthropogenic enhancement-environmental response. Global Biogeo. Cycles, 9, 235-252.

Gorselska, K. and J. N. Galloway. 1990. Amine nitrogen in the atmospheric environment over the North Atlantic Ocean. Global Biogeochemical Cycles 4 (3): 309-333.

Gorzelska, K., J.N. Galloway, K. Jackson, and W.C. Keene. 1992. Water soluble primary amine compounds in rural continental precipitation. Atmos. Environ., 26A, 1005-1018.

Gorzelska, K., J.R. Scudlark, and W.C. Keene. 1997. Dissolved Organic Nitrogen in the Atmospheric Environment. Chapter 20. In: Atmospheric Deposition of Contaminants to the Great Lakes and Coastal Waters. (Baker, J. E., Ed) SETAC Technical Publication Series, SETAC Press, pp. 379-392.

Gorzelska, K., R.W. Talbot, K. Klemm, B. Lefer, O. Klemm, G.L. Gregory, B. Anderson, and L.A. Barrie. 1994. Chemical composition of atmospheric aerosol in the troposphere over the Hudson Bay lowlands and Quebec-Labrador regions of Canada. J. Geophys. Res., 99, 1763-1779.

Graedel, T. E., D. T. Hawkins, and L. D. Claxton. 1986. Atmospheric Chemical Compounds: Sources, Occurrence, and Bioassay. Academic Press, Inc., Orlando, FL.

Grier, C.C., D.W. Cole, C.T. Cyrness, and R.L. Fredriksen. 1974. Nutrient cycling in 37 and 450 year old Douglas-fir ecosystems. Integrated Research in the Coniferous Forest Biome, (ed. R.H. Waring), 21-34. Coll. Forestry Res. Bull. 5.

Hannigan, M.P, G.R. Cass, A.L. Lafleur, J.P. Longwell and W.B. Thilly. 1994. Bacterial mutagenicity of urban organic aerosol sources in comparison to atmospheric samples. Environ. Sci. Technol. 28:2014-2024.

Hildemann, L. M., M. A. Mazurek, G. R. Cass, and B. R. T. Simoneit. 1991. Quantitative characterization of urban sources of organic aerosol by high-resolution gas chromatography. Environ. Sci. Technol. 25: 1311-1325.

IPCC 1996. Intergovenmental Panel on Climate Change, 1995 – The Science of Climate Change : Contribution of Working Group I to the Second Assessment Report of the IPCC. 576pp. Cambridge University Press, Cambridge, U.K.

Jordan, T.E., Correll, D.L., Weller, D.E. & Goff, N.M. 1995. Temporal variation in precipitation chemistry on the shore of the Chesapeake Bay. Water, Air, Soil Poll. 83, 263-284.

Kelley, J.M. and J.F. Meagher. 1998. Nitrogen input/output relationships for three forested watersheds in eastern Tennessee. 360-391.

Kirchman, D.L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Handbook of Methods in Aquatic Microbial Ecology (eds. P. Kemp, B. Sherr, E. Sherr and J. Cole), Lewis, pp. 509-512.

Likens, G.E., E.S. Edgerton, and J.N. Galloway. 1983. The composition and deposition of organic carbon in precipitation. Tellus, 35B, 16-24.

Lunde, G., J. Gether, N. Gjos, and M.-B. Stobet Lande. 1977. Organic micropollutants in precipitation in Norway. Atmos. Environ., 11, 1007.

Mazurek, M. A., G. R. Cass, and B. R. T. Simoneit. 1989. Interpretation of high-resolution gas chromatography and high-resolution gas chromatography/mass spectrometry data acquired from atmospheric organic aerosol samples. Aerosol Science Technology 10: 408-420.

Mazurek, M. A., G. R. Cass, and B. R. T. Simoneit. 1991. Biological input to visibility-reducing aerosol particles in the remote arid southwestern United States. Environ. Sci. Technol. 25: 684-694.

Mazurek, M.A., L.M. Hildemann, G.R. Cass, B.R.T. Simoneit, and W.F. Rogge. 1993. Methods of analysis for complex organic aerosol mixtures from urban emission sources of particulate carbon. In: Measurement of Airborne Compounds: Sampling, Analysis, and Data Interpretation. (Winegar, E. D. and Keith, L. H., Eds) American Chemical Society Symposium Series, CRC Press, Inc., Boca Raton, FL, 177-190.

Mazurek, M. A. and B. R. T. Simoneit. 1986. Organic components in bulk and wet-only precipitation. Critical Reviews in Environmental Control 16: 1-140.

Mazurek, M.A., B.R.T. Simoneit, G.R. Cass, and H.A. Gray. 1987. Quantitative high-resolution gas chromatography and high-resolution gas chromatography/mass spectrometry analyses of carbonaceous fine aerosol particles. Int. J. Environ. Anal. Chem 29: 119-139.

Mopper, K. and R. G. Zitka. 1987. Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling. Nature 325: 246-249.

Munger, J.W., Song-M. Fan, P.S. Bakwin, M.L. Goulden, A.H. Goldstein, A.S. Colman, and S.C. Wofsy. 1998. Regional budgets for nitrogen oxidess from continental sources: variations of rates for oxidation and deposition with season and distance from source regions. J. Geophys. Res., in press.

Munger, J.W., S.C. Wofsy, P.S. Bakwin, Song-M. Fan, M.L. Goulden,B.C. Daube, A.H. Goldstein, K.E. Moore, and D.R. Fitzjarrald. 1996. Atmospheric deposition of reactive nitrogen oxides and ozone in a temperate deciduous forest and a subarctic woodland 1. measurements and mechanisms. J. Geophys. Res., 101, 12,639-12,657.

NARSTO Synthesis Team. 2000. An assessment of tropospheric ozone pollution: A North American perspective.

National Acid Precipitation Assessment Program. 1991. Acid Deposition: State of Science and Technology, Volume 1. Emissions, Atmospheric Processes, and Deposition, U.S. National Acid Precipitation Assessment Program, Washington, D.C.

National Atmospheric Deposition Program/National Trends Network (1996). NADP/NTN Wet Deposition in the United States 1995. Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO, 11 pgs.

National Research Council 1993. Managing Wastewater in Coastal Urban Areas. National Academy Press., Washington, DC. 477 pp.

National Research Council 1993. Committee on Haze in National Parks and Wilderness Areas. Protecting Visibility in National Parks and Wilderness Areas. National Academy Press, Washington, D.C.

North American Research Strategy for Tropospheric Ozone (NARSTO). 1996. NARSTO-Northeast 1995 Summer Ozone Study. Report prepared by P. Roberts, M. Korc, D. Blumenthal, and P. K. Mueller, May 1996.

Paerl, H.W., J. Rudek, and M.A. Mallin. 1990. Stimulation of phytoplankton production in coastal waters by natural rainfall inputs: nutritional and trophic implications. Mar. Biol. 107:247-254.

Perciasepe, R. 2000. Mitigating atmospheric nitrogen inputs. Symposium on Nutrient Over-Enrichment of Coastal Waters: Global Patterns of Cause and Effect, Washington, DC, October.

Peterjohn, W.T. and D.L. Correll. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 65(5): 1466-1475.

Pierels, B.L. and Paerl. H.W. 1997. Bioavailability of atmospheric organic nitrogen deposition to coastal phytoplankton. Limnol. Oceanogr. 42:1819-1823.

Richter, D.D., C.W. Ralston, and W.R. Harms. 1983. Chemical composition and spatial variation of bulk precipitation at a coastal plain watershed in South Carolina. Water Resources Research 19: 134-140.

Riekert, H. 1983. Environmental impacts of intensive silviculture in Florida. Ballard, R. Symposium on Forest Site and Continuous Productivity, U.S. Dept. Agric. General Technical Report PNW-163, 264-271.

Rogge, W. F., M. A. Mazurek, L. M. Hildemann, G. R. Cass, and B. R. T. Simoneit. 1993e. Quantification of organic aerosols at a molecular level: Identification, abundance and seasonal variation. Atmos. Environ., 27A: 1309-1330.

Rozinoyer, I.M., A.P. Saprykina, and S.M. Poroyskaya. 1973. Organic matter in atmospheric precipitation in the vicinity of the City of Voronezh. Gidrobiol. ZH., 2, 100.

Saxena, P. and L.M. Hildemann. 1996. Water-soluble organics in atmospheric particles: A critical review of the literature and application of thermodynamics to identify candidate compounds. J. Atmos. Chem., 24, 57-109.

Seitzinger, S.P. and R.W. Sanders. 1997. Biologically reactive dissolved organic nitrogen inputs from rivers to estuaries. Mar. Ecol. Progr. Ser. 159:1-12.

Seitzinger, S.P. and R.W. Sanders. 1999. Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton. Limnology Oceanography 44: 721-730.

Seitzinger, S.P., R.W. Sanders, and R. Styles 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnology and Oceanography **47**: 353-366.

Seitzinger, S.P., R.M. Styles, R. Lauck and M.A. Mazurek. 2003. Atmospheric pressure mass spectrometry: a new analytical chemical characterization method for dissolved organic matter in rainwater. *Environ. Science Technol.* 37: 131-137.

Seitzinger, S.P., H. Hartnett, R. Lauck, M. Mazurek, T. Minegishi, G. Spyres, R. Styles. 2005. Molecular level chemical characterization and bioavailability of dissolved organic matter in streamwater using ESI mass spectrometry. *Limnology & Oceanography* 50(1): 1-12.

Semenov, A.D., L.I. Nemtseva, T.S. Kiskinova, and A.P. Pashanova. 1967a. Content of individual groups of organic substances in atmospheric precipitation. Gidrokhim. Mater., 42, 17.

Semenov, A.D., L.I. Nemtseva, T.S. Kiskinova, and A.P. Pashanova. 1967b. Organic substances in atmospheric precipitation. Dokl. Akad. Nauk SSSR, 173, 1185.

Sharp, J.H., R. Benner, L. Bennett, C.A. Carlson, R. Dow and S.E. Fitzwater. 1993. Re-evaluation of high temperature combustion and chemical oxidation measurements of dissolved organic carbon in seawater. Limnol. Oceanogr. 38: 1774-1782.

Shepson, P. B., E. Mackay and K. Muthuramu. 1996. Henry's Law constants and removal processes for several atmospheric beta-hydroxy and alkyl nitrates. Environ. Sci. Technol. 30:3618-3623.

Sidle, A.B. 1967. Amino acid content of atmospheric precipitation. Tellus, 19, 128.

Swank, W.T. and J.B. Waide. 1987. Characterization of baseline precipitation and stream chemistry and nutrient budgets for control watersheds. Forest Hydrology and Ecology at Coweeta, (ed. W.T. Swank), Springer-Verlag, 57-80.

Timperley, M.H., R.J. Vigor-brown, M. Kawashima, M Ishagami. 1985. Organic nitrogen compounds in atmospheric precipitation: their chemistry and availability to phytoplankton. Can.J. Fish. Aquat. Sci. 42:1171-1177.

United States Environmental Protection Agency. 1982. Chesapeake Bay program technical studies: A synthesis. Washington, DC, U.S. Environmental Protection Agency.

U.S. Environmental Protection Agency. 1996. National Air Quality and Emissions Trends Report, 1995. EPA-454/R-96-005, 168 pages.

U.S. Environmental Protection Agency. 1997. Nitrogen Oxides: Impacts on Public Health and the Environment. EPA 452/R-97-002. Office of Air and Radiation, Washington, DC, August 1997. 164 pages.

Vitousek, P. and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochem. 13:87-115.

Walsh, T.W. 1989. Total dissolved nitrogen in seawater: a new high-temperature combustion method and a comparison with photo-oxidation. Mar. Chem. 26:295-310.

Wedin, D.A. and D. Tilman. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science, 274, 1720-1723.

Willey, J.D., R. J. Kieber, M.S. Eyman, and G.B. Fr. Avery. 2000. Rainwater dissolved organic carbon: concentrations and global flux. Global Biogeochem. Cycles 14:139-148.

Appendix A

ESI response across a range of FV is repeatable and compound specific

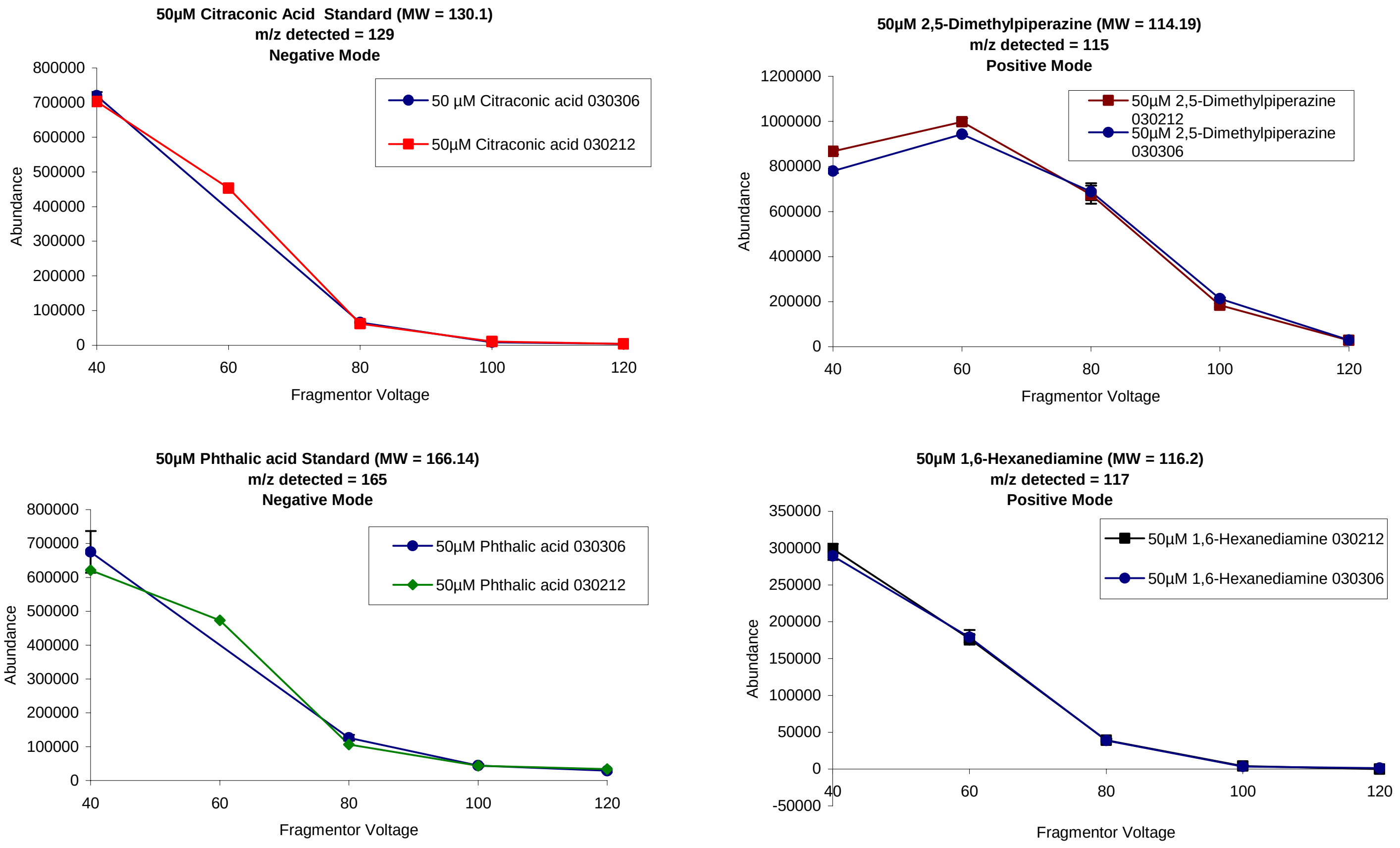
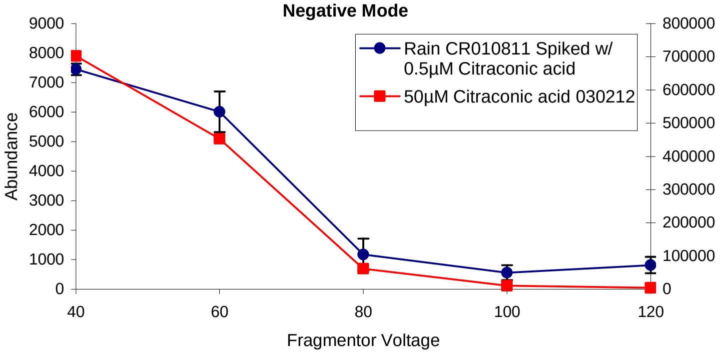
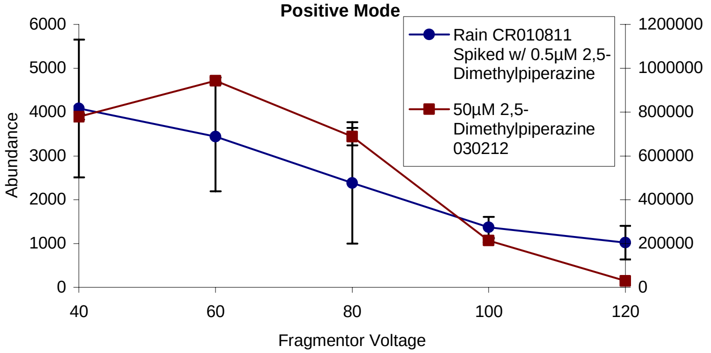
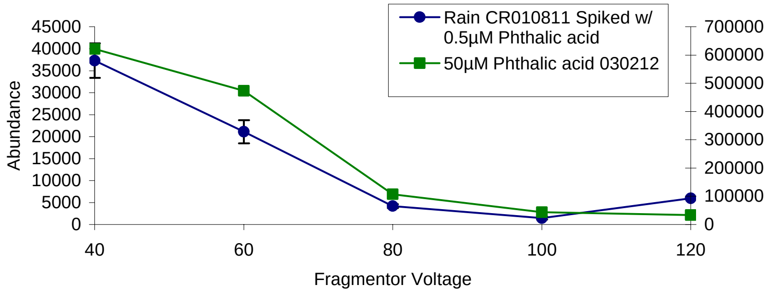


Figure A-1. The ESI-MS response across a range of ESI fragmentor voltages is compound specific and is repeatable.
The response to 4 standard compounds (citraconic acid, phthalic acid, 2,50dimethylpiperazine, and 1,6-hexaniediamine is shown.
Results from analysis on 2 separate days demonstrate the repeatability of results.

Spiked samples show the same ESI response across a range of FV as standards (few matrix effects)


Rainwater Sample CR010811 Spiked to 0.5µM increase of Citraconic acid 030212 (MW = 130.1)
m/z detected = 129
Negative Mode


Rainwater Sample CR010811 Spiked to 0.5µM increase of 2,5-Dimethylpiperazine (MW = 114.19)
m/z detected = 115
Positive Mode


Rainwater Sample CR010811 Spiked to 0.5µM increase of Phthalic acid 030212 (MW = 166.14)
m/z detected = 165
Negative Mode

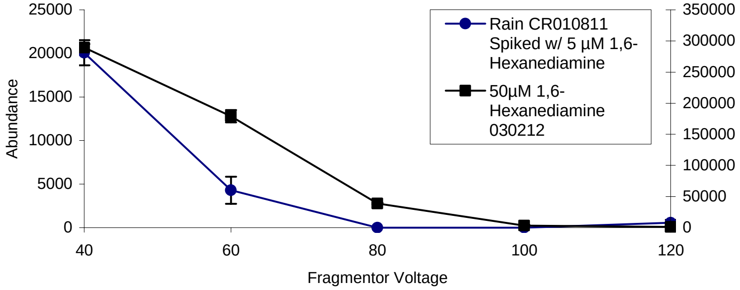

Rainwater Sample CR010811 Spiked to 5µM increase of 1,6-Hexanediamine (MW = 116.2)
m/z detected = 117
Positive Mode

Figure A-2. The ESI-MS response of samples spiked with known compounds is generally similar to the response to standards in deionized water. Some differences, particularly in the two positive mode standards were indicated. Shown are results from a Camden rain sample spiked with the 4 standard compounds.

Response of rainwater m/z 165 in 4 samples rainwater from Camden & Pinelands is similar to phthalic acid spiked samples across a range of ESI FV

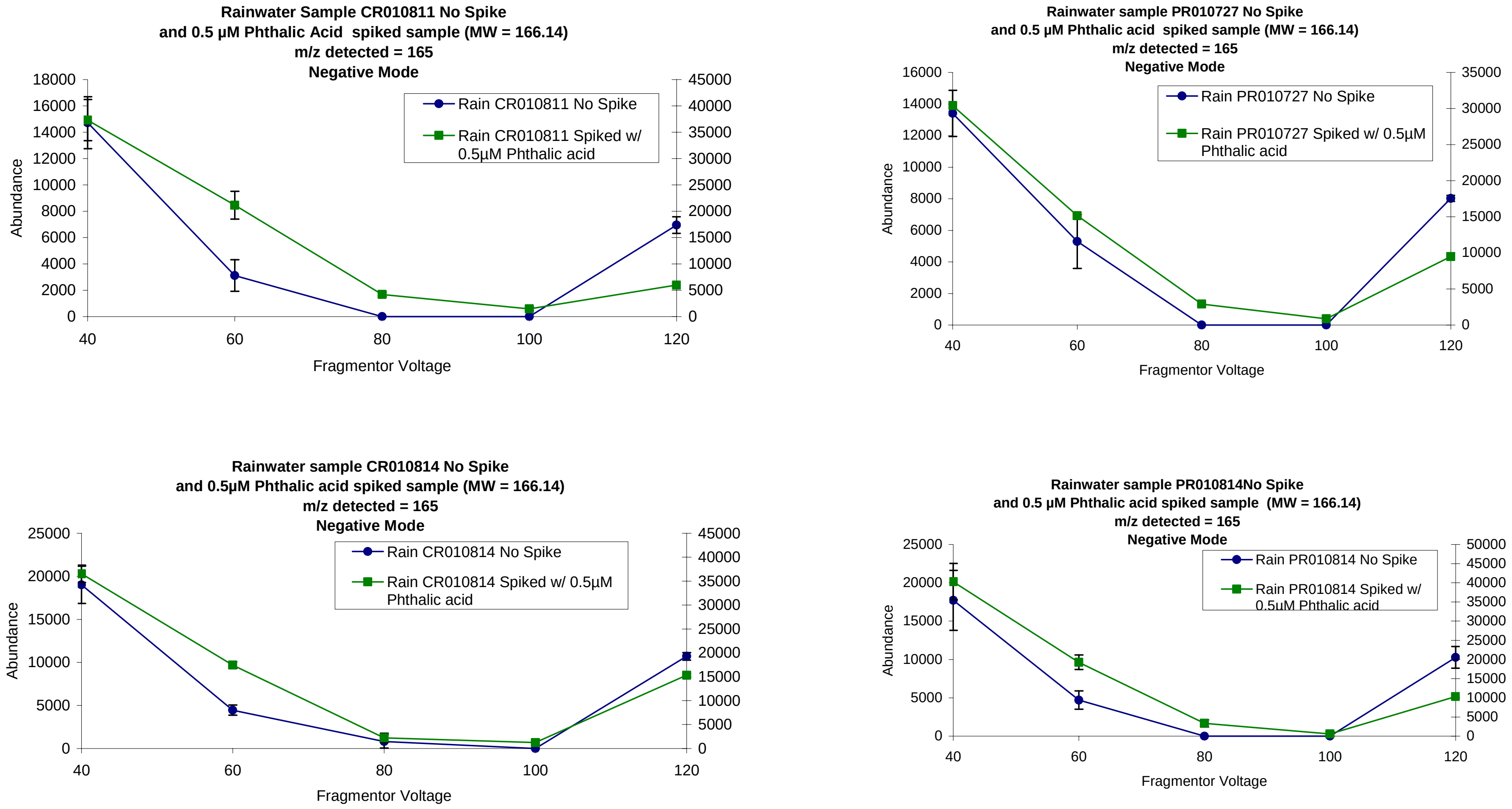
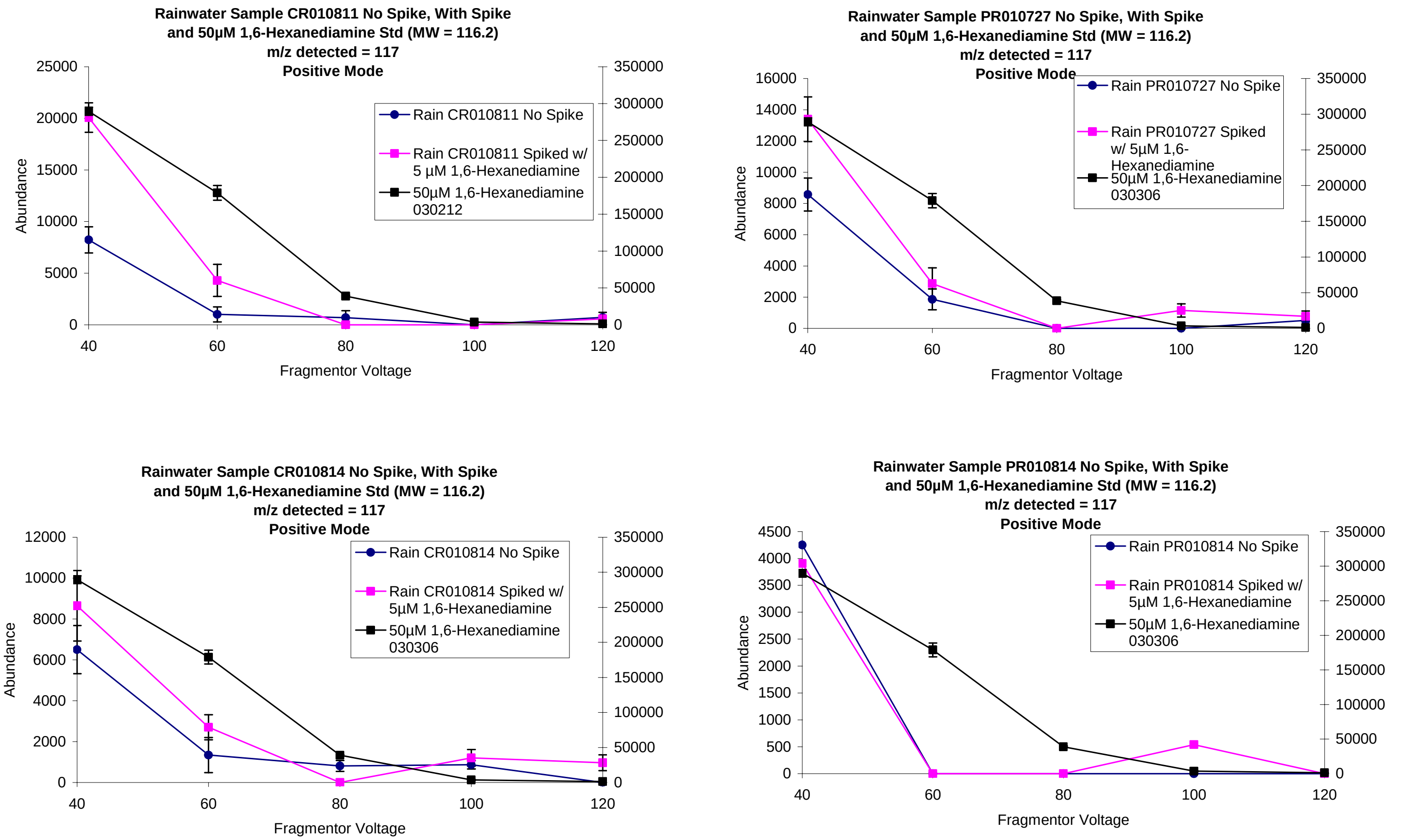


Figure A-3. The response of m/z 165 in two Camden and two Pinelands samples across a range of ESI fragmentor voltages is similar to the response to phthalic acid spiked samples, indicating that m/z 165 in these samples is phthalic acid. Y-axis on the right is for the spiked rain samples.

Response of rainwater m/z 117 in samples spiked with 1,6-Hexanediamine is similar for CR10811 only across a range of ESI FV.



Rainwater Sample CR010811 No Spike, With Spike and 50µM 1,6-Hexanediamine Std (MW = 116.2) m/z detected = 117 Positive Mode

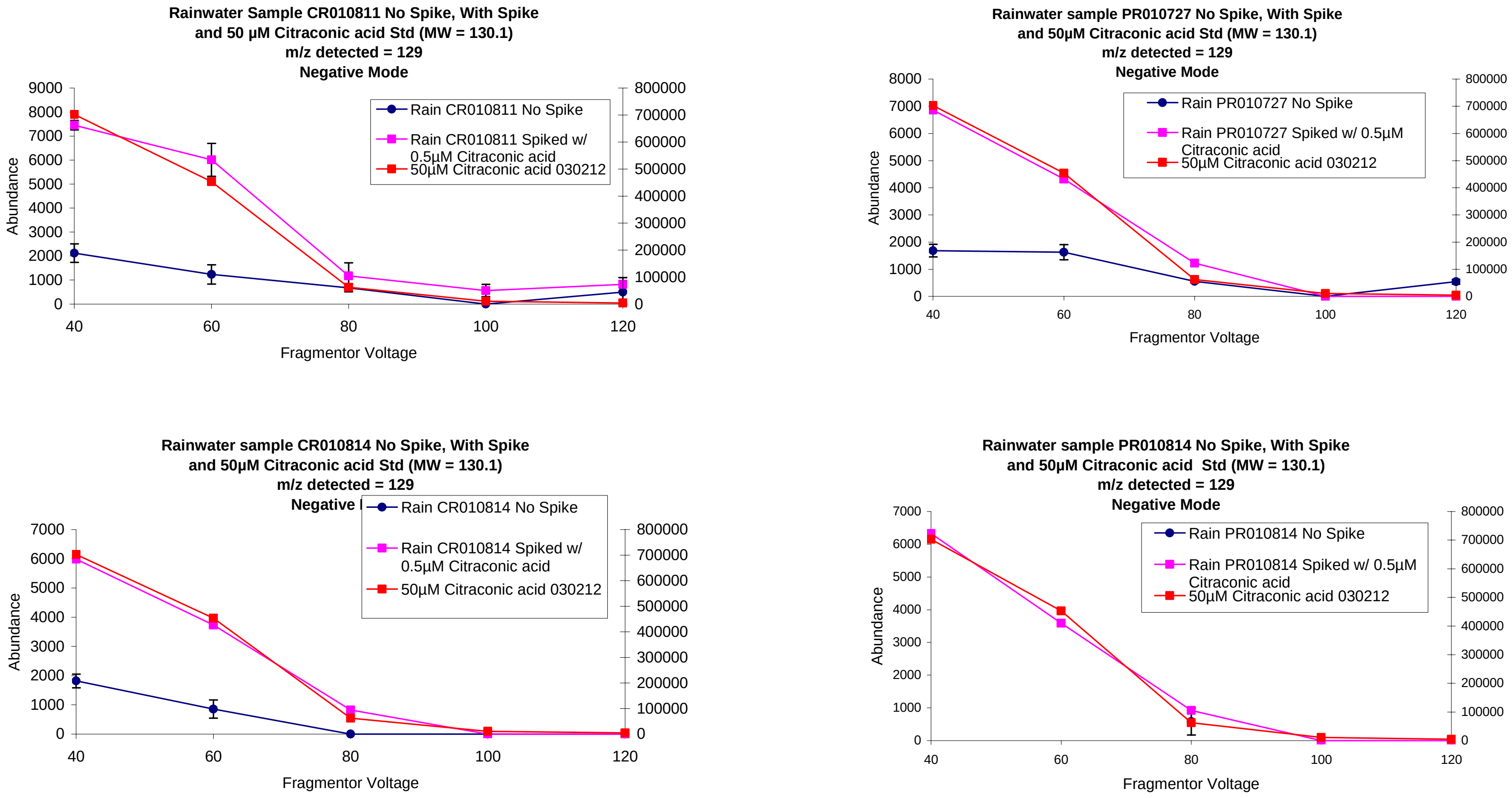
Rainwater Sample PR010727 No Spike, With Spike and 50µM 1,6-Hexanediamine Std (MW = 116.2) m/z detected = 117 Positive Mode

Rainwater Sample CR010814 No Spike, With Spike and 50µM 1,6-Hexanediamine Std (MW = 116.2) m/z detected = 117 Positive Mode

Rainwater Sample PR010814 No Spike, With Spike and 50µM 1,6-Hexanediamine Std (MW = 116.2) m/z detected = 117 Positive Mode

Figure A-4. The response of m/z 117 across a range of ESI fragmentor voltages in two Camden and two Pinelands samples compared to the response in the samples spiked to 5 µM 1,6-hexanediamine. The response is very similar for Camben sample CR10811, but shows some differences in the other 3 samples. Y-axis on the left is for the unspiked and spiked rain samples, and the Y-axis on the right is for the 50 µM 1,6-hexanediamine standard.

Response of rainwater m/z 129 in rainwater from Camden and Pinelands shows some similarities to citraconic acid across a range of ESI FV



Rainwater Sample CR010811 No Spike, With Spike and 50 µM Citraconic acid Std (MW = 130.1) m/z detected = 129 Negative Mode

Rainwater sample PR010727 No Spike, With Spike and 50µM Citraconic acid Std (MW = 130.1) m/z detected = 129 Negative Mode

Rainwater sample CR010814 No Spike, With Spike and 50µM Citraconic acid Std (MW = 130.1) m/z detected = 129 Negative Mode

Rainwater sample PR010814 No Spike, With Spike and 50µM Citraconic acid Std (MW = 130.1) m/z detected = 129 Negative Mode

Figure A-5. The response of m/z 129 in two Camden and one Pinelands samples across a range of ESI fragmentor voltages shows some similarities, but also a number of differences to the response to a citraconic acid standard, and to 0.5 µM citraconic acid spiked samples. In Pinelands sample PR010727, the m/z 129 was not detected and therefore no response in the unspiked sample was observed, although citraconic acid was detected when the sample was spiked with citraconic acid.

Response of rainwater m/z 115 in rainwater samples from Camden & Pinelands is similar for only 1 sample spiked with 2,5-Dimethylpiperazine

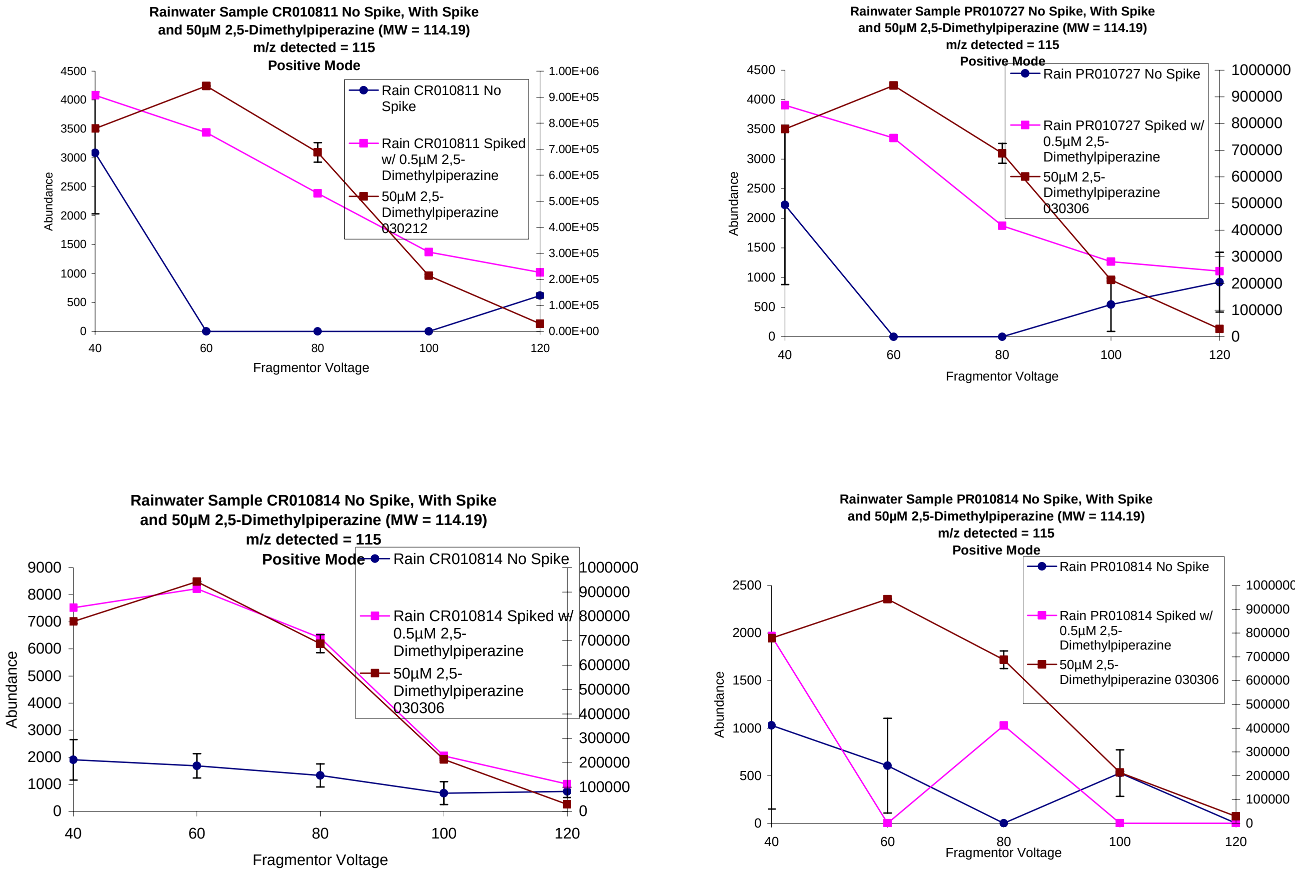


Figure A-6. The response of m/z 115 in two Camden and two Pinelands samples across a range of ESI fragmentor voltages differs in all but one samples (CR010814) from the response to 2,5-dimethylpiperazine spiked sample (spiked to 0.5 µM) and from that standard in deionized water, indicating that m/z 115 in those 3 samples is not 2,5-dimethylpiperazine; m/z 115 in the Camden samples (CR010814) may be 2,5-dimethylpiperazine. Y-axis on the left is for the unspiked and spiked rain samples, and the Y-axis on the right is for the 50 µM 2,5-dimethylpiperazine.

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