Using Nitrogen and Oxygen Isotopes to Locate Pollution from Non-point Sources in the Chesapeake Bay

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ABSTRACT

Locating non-point sources of pollution is essential to keep bodies of water (streams, rivers, lakes, groundwater, etc.) clean and to protect the humans, plants, and animals that depend on this water. However, cleaning up pollution after it has entered waterways is not a sustainable practice. Thus, the pollution must be managed at the source. Non-point sources of pollution have no obvious source which makes them particularly destructive to the environment. One specific location that is greatly affected by pollution is the Chesapeake Bay watershed. Nitrogen and oxygen isotope analysis has been proposed as an effective marker to pinpoint these non-point sources. One of the first challenges is determining the type of pollution (fertilizers, manure, chemical waste, etc.) in a particular waterway. A common form of non-point source pollution that is particularly difficult to locate is fertilizer. Many common types of fertilizer have been studied thoroughly.[1,2] Being able to differentiate between types of fertilizers can be the first step to tracing non-point sources back to where they originated. However, there has been no study to create a baseline of isotope abundance in the Chesapeake Bay which is required to locate non-point sources. Additionally, only nitrogen isotopes have been studied in depth within many types of fertilizers. Oxygen isotopes in water and deuterium isotopes can be beneficial to study which as well can further differentiate between types of pollution. Overall, utilizing nitrogen and oxygen isotopic abundance can be the first step to locating harmful sources of pollution in the Chesapeake Bay.

Introduction

Water is essential to every aspect of our lives and keeping it safe and clean is vital to a healthy environment. Clean water is crucial because of its applications in "irrigation, industry, transportation, recreation, fishing, hunting, support of biodiversity, and sheer esthetic enjoyment."[3] Pollution, however, causes major problems and has destroyed and impacted every body of water in one form or another. Only 3% of the earth is made of freshwater and even less of that is useable because of the impact of pollution. This puts additional stress on areas in drought and can escalate tensions where violence has already occurred over the ownership and usage of clean water. Thus, locating, cleaning, and preventing pollution is vital to every aspect of our lives.

Pollution can be categorized into two main types: point source and non-point source pollution. The birthplace of point source pollution can be easily determined such as a pipe leaking chemicals from a factory into a river or an oil spill from a wrecked tanker truck on a highway. These, though still potentially devastating, are much less impactful to the environment because they can be easily located which aids in containment and cleaning. Non-point source pollution, however, cannot be as easily located. One common form of non-point source pollution is the runoff of fertilizer and animal manure into rivers, streams, bays, etc. This can cause rapid growth in algae (called algal blooms) because of the surplus of nutrient runoff. When algae run out of these nutrients, however, they quickly die. The bacteria that decompose the dead algae require oxygen, resulting in oxygen-depleted zones that can kill surrounding plants and animals. These blooms can also "trigger spikes in pH levels, stressing fish, and create conditions that spur the growth of parasites."[4] Another harmful effect that agricultural pollution can have is that it can release harmful bacteria into

lakes and rivers. The pathogens and other bacteria present in manure can cause gastrointestinal issues and even death if not treated.[5]

Agricultural runoff after a large rain could arise from any number of nearby farms which makes locating the culprit of this pollution difficult. Simply cleaning up rivers and streams after runoff has reached them is not sustainable or cost-effective. Thus, locating the source of this runoff is crucial to protecting our waterways. To solve this problem, an analysis of the isotopic abundance of $\delta^{15}N$, $\delta^{17}O$, and $\delta^{18}O$ in varying types of fertilizer is being considered.[1,2] Because of the global increase in nitrogen-containing fertilizers,[6] this type of analysis will become crucial in determining non-point sources. These analyses have been studied before to locate point and non-point sources,[7,8] and, in addition to fertilizer, these isotopes have been used in stormwater runoff,[9] ammonium nitrogen, soil nitrogen, manure, and sewage analyses.[10]

Bateman[1,11] has done extensive research in identifying the different ^{15}N isotope concentrations in many types of fertilizer (**Table 1**). Not only did they test different types of fertilizers, but they also analyzed different brands of the same fertilizers to determine the difference in isotopic abundance between manufacturers. Additionally, they further cataloged fertilizers based on what is allowed in organic cultivation systems (**Table** 2). Thus, they can differentiate between farmers who use fertilizer for organic cultivation systems and those who do not. Michalski[2] goes further to analyze the isotopes of ^{15}N, ^{17}O, and ^{18}O (**Table 3**). Knowing what type and brand of fertilizer have been polluting bodies of water can be an essential aspect when determining the sources of pollution. Then, the challenge of locating the farmers who use the fertilizer in question and assisting them with preventative measures begins.

Table 1. Fertilizer composition and nitrogen isotope composition of synthetic nitrogen fertilizers. The fertilizer manufacturer is shown where known.[1]

	Fertilizer type	Manufacturer	$\delta^{15}N(‰)_{air}$
Synthetic	$(NH_4)_2SO_4$	W.L. Dingley	0.8
	$(NH_4)_2SO_4$	Gem	6.6
	$(NH_4)_2SO_4$	Terra	-1.2
	$(NH_4)_2SO_4$	Bunn	0.7
	KNO_3	W.L. Dingley	-1.5
	KNO_3	Gem	-1.1
	KNO_3	Yara	-1.0
	Urea	Gem	-2.4
	Urea	Unknown	-1.1
	Urea	Bunn	-0.8
	Urea	W.L. Dingley	-1.6
	Urea	Yara	-5.9
	NH_4NO_3	Unknown	2.6
	NH_4NO_3	Bunn	0.5
	NH_4NO_3	Kemira	2.2
	NH_4NO_3	Terra	-1.3
	NH_4NO_3	Yara	-1.4
	NH_4NO_3	Unknown	-0.9
	$(NH_4)H_2PO_4$	Gem	-0.9
	$(NH_4)H_2PO_4$	Terra	-0.3
	NPK 20-10-10	Kemira	1.9
	NP 27-10	Kemira	0.8
	NPK 28-5-5	Kemira	1.1
	$Ca(NO_3)_2$	Yara	-0.3
	NPK 16-16-16	Yara	-0.6

NPK 21-8-11	Yara	-0.7
NPK 12-12-12	Unknown	0.4
Hydroponic solution NH_4NO_3 and $Ca(NO_3)_2$	Unknown	0.2
Hydroponic solution KNO_3 and $Ca(NO_3)_2$	Unknown	0.7

Table 2. Nitrogen isotope composition of fertilizers that may be permitted in organic cultivation systems. The fertilizer manufacturer is shown where known. Some fertilizers were supplied from 'Growers' and this is indicated on the table.[1]

	Fertilizer type	Source	$\delta^{15}N(‰)_{air}$
Manure/composts	Farmyard manure	Grower	8.5
	Farmyard manure	Grower	9.3
	Farmyard manure	Grower	3.5
	Farmyard manure	Grower	7.2
	Farmyard manure	Grower	14.1
	Farmyard manure	Grower	16.2
	Farmyard manure + compost	Grower	4.9
	Farmyard manure	Grower	6.9
	Chicken manure pellets	Rooster	5.4
	Chicken manure pellets	Westland	4.8
	Chicken manure pellets	Grower	8.4
Seaweed based	Seaweed extract	Grower	1.5
	Seaweed extract	Maxicrop	1.3
	Seaweed based liquid feed	Greenfingers	2.9
	Seaweed based liquid feed	Vitax	3.1
	Natural seaweed meal	Maxicrop	5.4
	Seaweed based liquid feed, Type 1	B&Q	0.6
	Seaweed based liquid feed, Type 2	B&Q	1.7
	Seaweed based fertilizer, Type 1	Grower	3.7
	Seaweed based fertilizer, Type 2	Grower	2.1
Mammalian/non manure	Dried blood	W.L. Dingley	4.1
	Dried blood	Gem	6.8
	Dried blood	J. Arthur Bowers	6.6
	Dried blood	Vitax	6.3
	Hoof and horn	W.L. Dingley	6.2
	Hoof and horn	Gem	6.6
	Hoof and horn	Grower	6.3
	Bonemeal	Gem	5.1
	Bonemeal	J. Arthur Bowers	4.7
Fish based	Fish, blood, and bone	J. Arthur Bowers	7.0
	Fishmeal	Grower	10.6
	Fishmeal-based	Nugro 8.7.7	2.1
	Fishmeal-based	Nugro 6.1.3	8.7

Table 3. Isotopic composition of commercial fertilizers, reagent-grade nitrate salts, nitric acid, and organic labeled fertilizer.[2]

Nitrate source	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\delta^{17}O$ (‰)	$\Delta^{17}O$ (‰)
Commercial fertilizers				
UAN (n = 270)	0.3	24.2 ± 3.0	11.4	-0.15 ± 0.30
$NH_4(NO_3)$ (12)	ND	28.8	14.7	-0.24 ± 0.027
Reagent-grade nitrate				
$Cu(NO_3)_2$	ND	22.8	11.2	-0.17 ± 0.040
$Mg(NO_3)_2$	ND	17.3	8.4	-0.019 ± 0.037
$Al(NO_3)_2$	ND	15.7	7.5	-0.096 ± 0.013
$Co(NO_3)_2$	ND	21.6	10.4	-0.25 ± 0.024
$Zn(NO_3)_2$	ND	23.9	11.9	-0.016 ± 0.04
$LiNO_3$	ND	23.0	11.3	-0.13 ± 0.045
KNO_3	ND	24.4	12.0	-0.14 ± 0.058
$Fe(NO_3)_3$	ND	20.4	10.1	0.040 ± 0.027
HNO_3 (10)	ND	25.7 ± 1.3	12.7	-0.22 ± 0.02
Organic fertilizers				
Bonide Nitrate of Soda	-1.5	49.7	44.7	18.9 ± 0.2
Hoffman Nitrate of Soda	0.5	52.6	47.2	19.8 ± 0.3
Drammatic One liquid	-4.4	50.5	45.3	19 ± 0.4
Hi-Yield Nitrate of Soda	-1.2	51.7	46.3	19.4 ± 0.3
USGS35	-2	57.5	51.5	21.1 ± 0.2

Notes: Standard deviations for the analysis of the same compound but different origins (UAN, NH_4NO_3, and HNO_3) represent the range of values for that compound. $\delta^{17}O$ standard deviation for metal nitrates reflects replicate analysis of the same sample. No $\delta^{15}N$ was determined for metal nitrates because the use of thermal decomposition produces O_2.

Impact of pollution on the Chesapeake Bay

Pollution is a worldwide problem, but this study will focus specifically on the Chesapeake Bay watershed. The impact of pollution on the Chesapeake Bay has been studied extensively since the 1960s and beyond.[12–15] Until the late 1900s, however, marine systems "were often considered invulnerable to effects of nutrient enrichment because of their well-mixed, well-flushed nature"[16] which allowed pollution to impact eutrophication in the Bay unchecked. Now "the problem of eutrophication has become a central theme of coastal research and management at regional and global scales"[14] because scientists quickly realized that all bodies of water are impacted heavily by pollution. Because of a lack of understanding of the impacts that pollution has on the Chesapeake Bay, the consequences have become more severe and impacted every part of the Bay and its watershed.

Main types of pollution in the Chesapeake Bay

The primary forms of pollution affecting the Chesapeake Bay are nitrogen and phosphorous. "Excessive nitrogen and phosphorus degrade the Bay's water quality. The majority of nitrogen and phosphorus pollution comes from sewage treatment plants, animal feedlots, and polluted runoff from cropland, urban, and suburban areas. In addition, air pollution (from vehicle exhaust) and industrial sources such as power plants contribute roughly 1/3 of the nitrogen

pollution."[4] Specifically, "agricultural activities are estimated to contribute approximately 44% percent of nitrogen and phosphorus loads, and 65% of the sediment loads delivered to the Bay, making agriculture the largest source of nutrients and sediments to the Bay."[17]

Impact of runoff and other contributing factors that affect non-point source pollution in the Chesapeake Bay

With agriculture comes manure runoff from animals and fertilizer runoff from crops. "Nutrient enrichment has contributed to widespread changes in coastal habitats, including loss of seagrasses, proliferation of harmful phytoplankton and benthic macroalgae, and depletion of dissolved oxygen in bottom waters."[14] "Manure is the source of 18% of the nitrogen and 27% of the phosphorus entering the Chesapeake Bay annually (**Figure 1**). The Chesapeake Bay Watershed is home to 3.2 million animal units (animal unit = 454 kg [1,000 lbs] of livestock) generating roughly 36 million t (40 million tn) of livestock manure per year."[18] If left unchecked, manure runoff would be a detrimental problem to the health of the Bay watershed. To combat this, the primary disposal method is to collect and reuse manure as fertilizer which lessens the load on the Bay. However, some regions of the Bay have areas where "manure nutrient production exceeds the assimilative capacity of the land."[19] **Figure 2** shows that most of Maryland, Delaware, and the coastal regions of Virginia and Pennsylvania have large nitrogen loads per square kilometer. Thus, during large rains, much of the excess nutrients are washed into the Bay due to poor runoff management and ineffective manure fertilization of crops.

Another issue is that different areas of the Chesapeake Bay contribute varying amounts of runoff into the watershed based on geography and location. Some regions are more forested while other regions are more urban. Forested regions provide natural buffers that can absorb excess nutrient runoff before it enters streams and rivers. Urban areas, however, have more runoff because they have less farmland to distribute manure and fewer buffer regions. To make the problem worse, there is a correlation between high nitrogen yields (**Figure** 2) and large populations (**Figure** 3) around the Bay. Overall, certain regions (and even certain farms in the same region) of the Bay contribute more pollution than others. Thus, locating these non-point sources of pollution is essential to the health of the Chesapeake Bay.
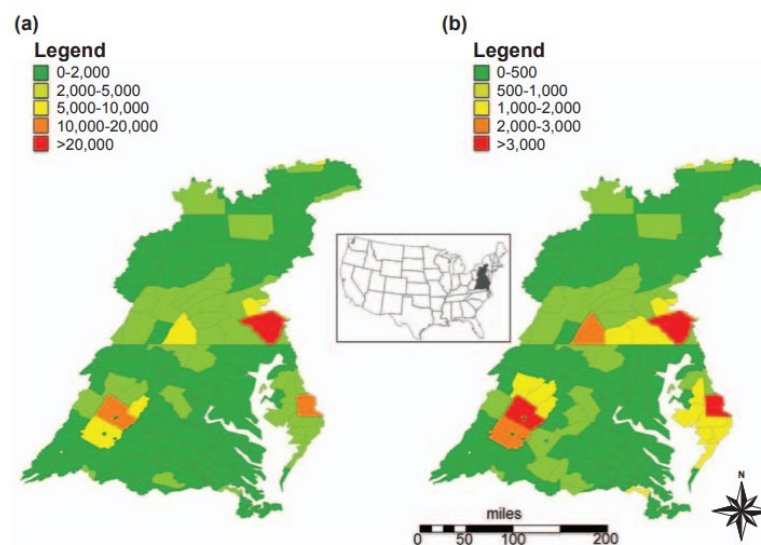

Figure 1. Annual production of manure nutrients (lbs) in 2009 in counties of the Chesapeake Bay Watershed. (a) Manure nitrogen. (b) Manure phosphorus. The location of the watershed is identified in the inset map of the United States.[18]
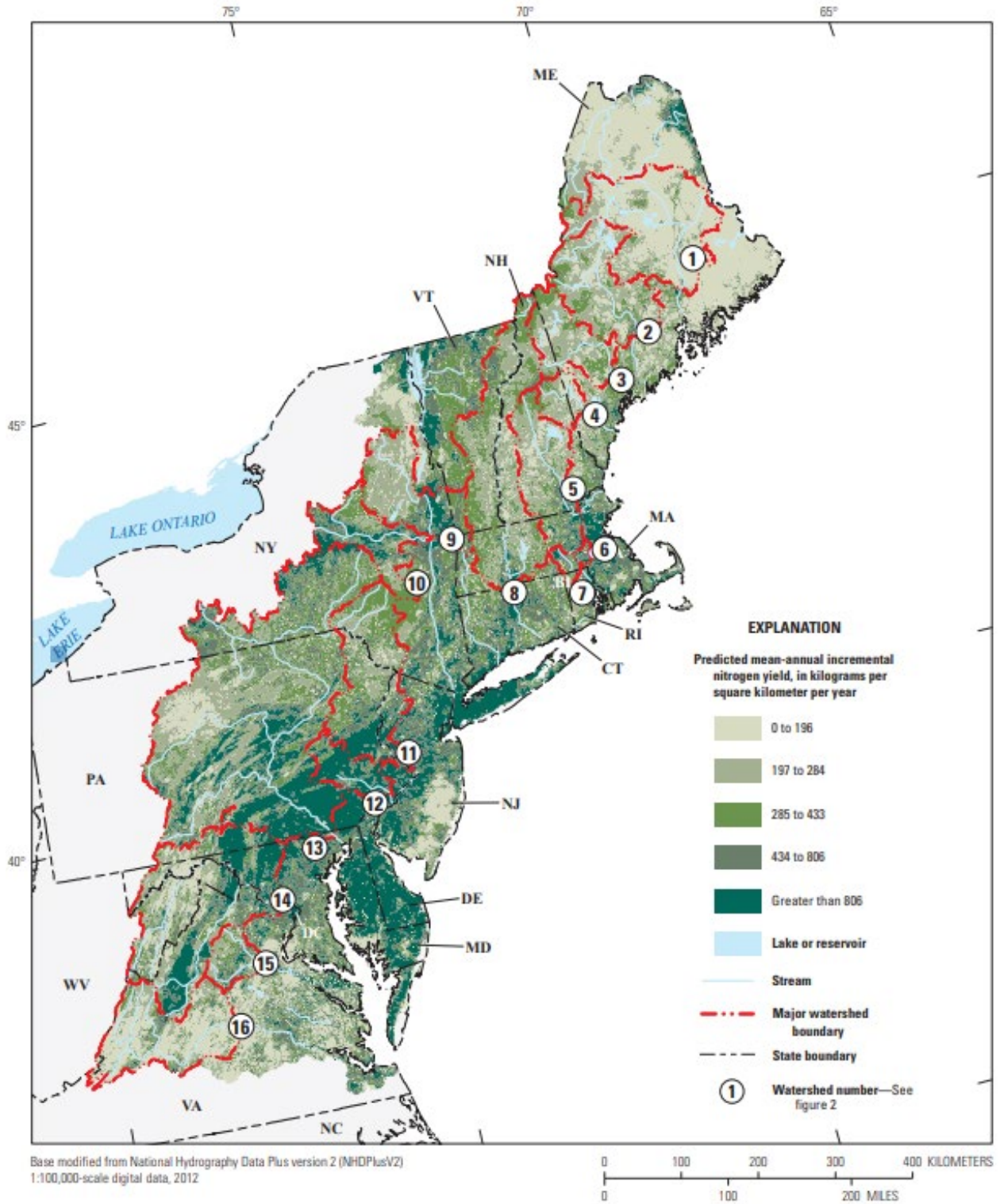
Figure 2. Predicted mean-annual incremental yield of nitrogen to streams in the Northeastern United States, 2012.[20]
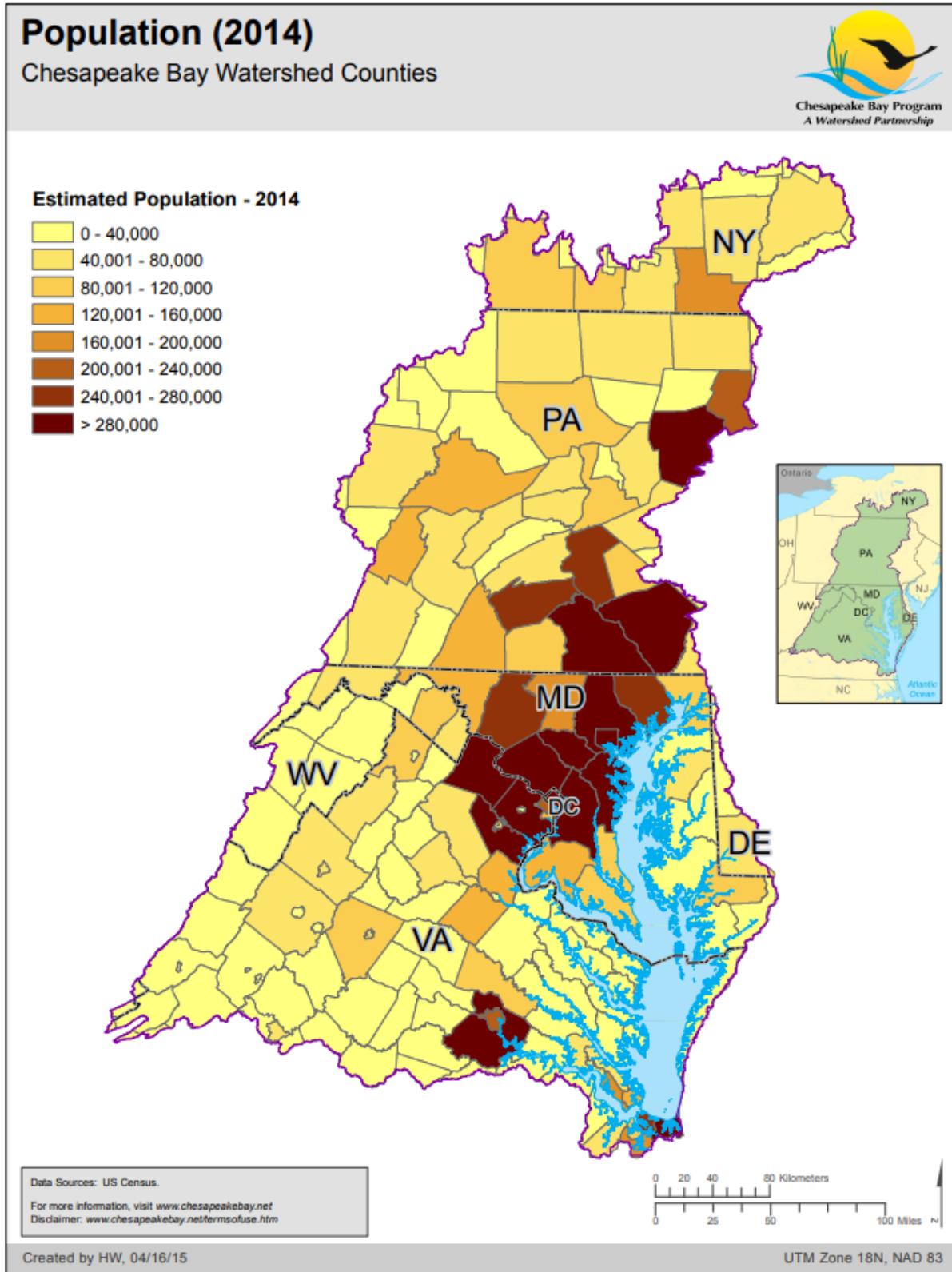
Figure 3. This map shows population estimates within the Chesapeake Bay watershed on a county-by-county basis for the year 2014. For counties not completely within the Chesapeake Bay watershed, the population numbers were derived as a proportion of the total county based on land area.[21]

δ¹⁵N, δ¹⁷O, and δ¹⁸O in the Bay

To locate these non-point sources, analyzing the nitrogen and oxygen isotopes has been proposed. δ¹⁵N is present in the air with a natural abundance of 0.366%.[22] The natural abundance of δ¹⁷O and δ¹⁸O is 0.038% and 0.205% respectively. The stable isotope compositions are expressed per mil (‰) relative to the international standard (**Equation 1**).

However, this percentage fluctuates during processes such as nitrification which can add low δ¹⁵N and uptake of NO₃⁻ or NO₂⁻ by phytoplankton which can increase the δ¹⁵N concentration.[23]

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000$$

Equation 1. The calculation for the stable isotope compositions is based on international standards "where the positive value and negative value of δ respectively represent the enrichment and impoverishment of heavy isotopes in the test sample compared with a standard sample."[10]

Another difficulty can include seasonal changes in isotope concentrations similar to the findings of the Daliao river basin in China according to Zhang.[24] However, no seasonal correlations have been found in the Chesapeake Bay in 1990 or 1998.[23,25] Horrigan states, "the δ¹⁵N of the (NO₃⁻ and NO₂⁻) pool can be affected by several different processes, and any seasonal pattern could be obscured by the effects of a variety of short-lived physical (e.g. vertical mixing) and biological (e.g. denitrification) events."[23] Thus, determining a baseline for natural, albeit polluted, isotope concentrations is required. Sigleo determined that the δ¹⁵N for suspended particles in the Chesapeake Bay and the Potomac River (the longest river that feeds into the Bay) to be +7·7±3·1‰ in 2002.[26] However, they go on to state that "the highest values occurred during summer when the primary source of nitrogen originated from remineralized organic material."[26] This is contrary to the findings stated by Horrigan[23] and Junk.[22] Furthermore, Li says in 2017, "The water column of the Chesapeake Bay exhibited strong spatial and seasonal variability in concentrations of dissolved oxygen and concentrations of inorganic nitrogen and phosphorus."[27] Potentially the short-lived physical and biological events mentioned by Horrigan[23] are enough to constitute a seasonal change. Another possibility is that they did not conduct thorough enough research to determine the impact of seasonal change in the late 1900s and early 2000s while the impact has been correctly analyzed in recent years. These contradictions of a seasonal impact would need to be analyzed further, and a more comprehensive study of the natural abundance of δ¹⁵N, δ¹⁷O, and δ¹⁸O will need to be conducted in the Bay.

Methods for quantifying N and O isotopes

To study the isotopic abundance, water samples must be taken at various points at differing depths in the Bay throughout the year for multiple years. This will ensure that short-lived changes in isotope concentrations can be ruled out to create a baseline for future analysis. Multiple techniques have been discussed on how to determine the isotopic concentration of ¹⁵N, ¹⁷O, and ¹⁸O. The most popular methods are the ion-exchange method,[28] denitrifier method,[29], and the Cd-azide reduction method.[10,30] The denitrifier method was further refined by Casciotti.[31] An additional method for analyzing the δ¹⁷O and δ¹⁸O concentrations include utilizing a Thermo Chemical Elemental Analyzer (TC/EA) coupled with a Delta V continuous flow isotope-ratio mass spectrometer.[27] However, "no single simple method has been able to automatically extract the nitrate in water samples and meanwhile to completely avoid the isotope contamination and fractionation."[10] This makes isotopic analysis difficult and time consuming since it cannot be conducted in the field.

How N and O isotopes can be used to locate non-point sources in the Bay

Once a baseline of isotopic concentrations has been determined, based on any of the methods above, the data can be compared to known isotopic abundance in common fertilizers (**Tables 1**, **2**, and **3**). For instance, if a certain area of the Bay has a $\delta^{15}N$ (‰)$_{air}$ of 6.6, that could correspond to dried blood made by J. Arthur Bowers or hoof and horn made by Gem (**Table 2**). With only this information, a survey of surrounding farms could be conducted to determine the source of the pollution. However, with only this information, a reliable categorization of where the fertilizer came from cannot be completed. A more detailed analysis of oxygen isotopes in fertilizers will need to be conducted before any major claims are made. Furthermore, it must be reliably determined that the nitrogen and oxygen isotopes found in the Chesapeake Bay are not from other sources of pollution (sewage, factory runoff, etc.). Thus, not only is a more in-depth study of fertilizer needed, but also a comprehensive study of the nitrogen and oxygen isotopes of other forms of pollutants will need to be conducted. This can ensure reliable results for the specific types of pollution in the Bay which can be traced back to the non-point sources. Once these locations of excess runoff are known, measures can be taken to prevent runoff in the future.

Conclusions

Overall, locating non-point sources is crucial to improving global water quality, and analyzing agricultural runoff is a key aspect of this goal. However, this is a very complex issue and will require extensive research. To start, there is no global database for isotopic information to compare to the Chesapeake Bay. "Until now, there is no review that systematically summarizes present limitations and future prospects of the application of nitrogen and oxygen isotopes… The lack of a global landscape map of nitrogen and oxygen stable isotopes of nitrate in surface water has greatly affected the accuracy of these results."[10]

Simply analyzing $\delta^{15}N$, $\delta^{17}O$, and $\delta^{18}O$ might not be enough to accurately determine the sources of pollution because, "the $\delta^{15}N$ values of nitrate from precipitation, soil, chemical fertilizer, manure, and sewage partly overlap"[10] (**Figure 4**). To solve this, additional studies can be conducted to include molar ratios of elements. For instance, Chen discovered that specific nitrate of surface water from fertilizer, animal manure, or sewage could be calculated by analyzing the molar ratio of NO_3^- to Cl^-.[32] Furthermore, Showers determined that septic tanks and manure can be differentiated by analyzing the δ^2H and $\delta^{18}O$-H_2O of septic tank effluent and groundwater.[33]

Many studies have been conducted regarding $\delta^{15}N$, $\delta^{17}O$, and $\delta^{18}O$. However, there needs to be a comprehensive study of their natural abundance in the Chesapeake Bay to utilize these isotopes to their fullest potential to locate non-point sources of pollution. There is hope, however, that after such a study(s) is conducted, we can make strides to further eliminate pollution in our waters.
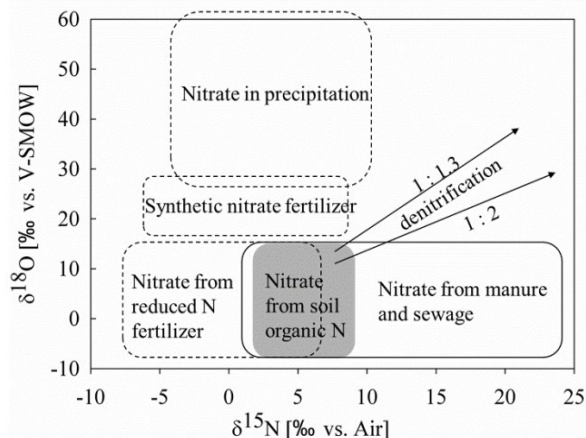

Figure 4. Distribution of δ 15N and δ18O from different potential nitrate sources[10]

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