Organic phosphate isotopes method offers a new way to track pollutants in the environment

September 4 2024, by Katie Peikes

Tony Hollenback, a recent Ph.D. graduate, uses a precision needle to inject the phytate molecule purified from soil into the Orbitrap IRMS. Credit: Kathy F. Atkinson

Plants need phosphorus, an essential nutrient, to grow. This nutrient is present in fertilizers and applied to crops to increase their yields. One downside of too much phosphorus in this agricultural environment is it can creep into lakes, rivers and other water bodies, causing water quality problems such as algae blooms, which remove oxygen from the water and create dead zones, thus killing fish and other aquatic life.

Phosphorus in the environment is present in two forms: organic and inorganic. Inorganic phosphorus is the simplest form and most readily accessible to soil microorganisms and plants. Organic phosphorus compounds are much more complex, making them difficult to identify and track in the environment.

Scientists often use an element's isotopes as a means to track its presence in the environment. This is because isotopes of an element have the same number of protons, but different numbers of neutrons.

They're basically families of the same element with slightly different masses. For example, carbon-13 is an isotope of carbon that has been useful in determining the age of groundwater, while carbon-14 has helped researchers date material artifacts, such as pottery, to a particular time period.

Scientists have long developed ways to measure isotopes of inorganic phosphate oxygen in the environment. But there is no such method to measure isotopes of organic phosphate.

"The existing method requires removing phosphate from a molecule," said Deb Jaisi, a University of Delaware professor of environmental biogeochemistry in the Department of Plant and Soil Sciences.

"Once you remove (hydrolyze) it, it is no longer organic and no longer can be used for source tracking because this change alters the original

isotopes of the molecule."

That's where Jaisi and Tony Hollenback, a recent Ph.D. graduate from Jaisi's lab, come in.

The duo has published new research in the *Journal of the American Society for Mass Spectrometry* detailing the development of a new method for measuring the isotope fingerprints of organic phosphate molecules using mass spectrometry techniques.

The method leverages a tool called an electrospray ionization-based Orbitrap isotope ratio mass spectrometer (Orbitrap IRMS), an advanced instrument designed for such analyses. UD is home to one of only nine such Orbitrap IRMS instruments nationwide.

Tracking where pollutants come from

Studying where environmental contamination comes from is necessary if researchers hope to come up with solutions for eradicating the contamination, identifying best management practices, and/or cleaning up a contaminated site.

In the Chesapeake Bay, the watershed Hollenback and Jaisi took soil samples from, phosphorus is a major pollutant affecting the bay's health. The state of Maryland has a plan for how to reduce the bay's phosphorus by a certain amount by 2025.

Phosphorus can degrade water quality through triggering algal blooms. The algal blooms suck oxygen out of the water, killing fish, plants and other organisms.

"Simply put, we want to know where the phosphorus is coming from," Jaisi said.

Jaisi and Hollenback looked at one molecule, phytates, in particular. All plant seeds that are fed to pigs and chickens, on the Delmarva Peninsula or anywhere else, are very high in phytate. Hollenback explained that pigs and chickens are "non-ruminants," which means they don't have certain enzymes to break down the phytates. This means phytate concentrates in the animals' manure.

"If that manure is applied to crop fields as fertilizer, phytate concentrates in the soil," Hollenback said. "During rain events, this phytate is then transferred to nearby streams, which then drain into the bay. Now there are microbes in the water, be it fungi or bacteria, and they do have enzymes to break it down."

When the fungi or bacteria break down the phytate, they release inorganic phosphate, the most bioavailable form of phosphorus, into the water, thus allowing harmful algae to grow.

"So we need to be able to track it," Hollenback said, "because it's potentially a very big puzzle piece in trying to fix this problem."

A new method

To do this, Hollenback and Jaisi developed a method for measuring organic phosphate isotopes using the Orbitrap IRMS. The experiment was done in UD's Patrick T. Harker Interdisciplinary Science and Engineering Laboratory.

The UD researchers collected soil from a farm outside of Crisfield, Md. near East Creek, a tributary of the Chesapeake Bay. This particular soil, which has been fertilized with animal manure for a long time, is rich in phytate, the organic phosphorus compound most commonly found in agricultural soils.

"The goal of this research was two-fold," Hollenback said. "One [goal] was to look at the soil biology, see what is interacting with the phytate. The other was to explore and track oxygen isotopes in phytate using the Orbitrap IRMS."

The researchers incubated the soil samples under laboratory conditions for about three months. They also "spiked the soil" with extra phytate, Hollenback said. This helped to see if any changes happened in the soil. They even added isotopically-labeled water to investigate how phytate would cycle through the system.

"A lot of the changes we saw with regard to the extra phytate formed were biological," Hollenback said. "A key finding of this research was that the phytate molecule faithfully preserves its isotope fingerprints."

The method using the Orbitrap MS originated at the California Institute for Technology, but that institution used it for other compounds, not phosphate. The UD researchers inject a solution containing purified phytate from soils into the instrument using a high-precision syringe.

The molecules travel through a mass filter which removes any contaminant ions. The mass is then measured very precisely in the Orbitrap mass analyzer by tracking an ion band as it revolves around a central spindle.

The aptly named Orbitrap MS "traps" ions to measure the molecules—both composition and isotopes. Since this measurement was made for isotopes, a new name Orbitrap IRMS (Orbitrap isotope ratio mass spectrometry) was coined.

"The isotope of phosphate in the phytate molecule was untouched by any processes," Jaisi said. "We found that the isotope's signature of the molecule remains the same."

That's a breakthrough. If the isotope of a molecule remains the same, it meets the first requirement to identify the source of a molecule or contaminant in the environment. If the isotope of a molecule were to change in any way, it would essentially erase the molecule's origin.

"Now we have a new wheel," Jaisi said. "A new wheel we've developed instrument-wise. And isotope-wise, since the molecule has a signature that remains the same, the finding can be used for source tracking for this compound and can be analogously used for other molecules."

The next phase is to see how it works in the real environment. The good news is that the actual field testings are underway.

Furthermore, the method Jaisi and Hollenback developed with the Orbitrap IRMS can be used across a variety of disciplines and chemical compounds. It's a big asset to UD, as other research teams at the university are already using the Orbitrap IRMS to study various compounds. Among them are explosive compounds and even "forever chemicals" known as PFAS, per-and polyfluoroalkyl substances.

"It's such a powerful instrument," Jaisi said. "While it's not very routine for anyone to be able to use it without specific training, methods are being continued to be optimized with the instrument. It's in the very early stage, but it's a great opportunity for anyone to jump in and develop leadership science using the instrument."

More information: Anthony J. Hollenback et al, Position-Specific Oxygen Isotope Analysis in Inositol Phosphates by Using Electrospray Ionization-Quadrupole-Orbitrap Mass Spectrometry, *Journal of the American Society for Mass Spectrometry* (2024). DOI: 10.1021/jasms.4c00210