

## HOW PLANTS AND BACTERIA CAN CLEAN THE EARTH

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### YOUNG REVIEWERS:



ALY  
AGE: 13



DARIO  
AGE: 15

### TRICHLORO-ETHYLENE

A chemical widely used as solvent and degreaser that is persistent in the environment as a pollutant.

Have you ever seen black smog come out of cars? Or noticed an unnatural oily film on water puddles in parking lots? Humans have polluted practically every corner of our planet and most of it is invisible to our eyes. Sadly, Earth suffers the consequences of this pollution. But scientists have recently found a simple way to restore contaminated soils back to health. In this article, we will talk about how plants and bacteria team up to restore our polluted world!

### WHAT IS REMEDIATION?

Years of irresponsible human activities have caused our planet to become polluted with harsh chemicals. Once these chemicals settle into the earth, it is very challenging to remove them. These pollutants pose a huge threat to the health of humans and wildlife. **Trichloroethylene** (TCE) is a long-lasting chemical pollutant that has seeped into soils and groundwater from manufacturing sites, where it

## ENVIRONMENTAL PROTECTION AGENCY

An agency of the US government responsible for protecting the environment.

## BIOREMEDIATION

Use of an organism's natural ability to remove and break down pollutants in the environment.

## PHYTOREMEDIATION

The use of a plant's natural ability to remove and break down environmental pollutants.

## ENDOPHYTE

Bacteria or fungi that live within a plant.

## SYMBIOTIC

A long-term interaction between two different organisms.

is used as a degreaser. TCE is known to cause cancer, which is why the **Environmental Protection Agency** from the United States considers it a top priority to clean up [1].

What are we doing about this problem? One popular method is excavation, in which contaminated soil is literally dug out of the ground with big construction machines. If the groundwater, the water layer below the soil surface, is polluted, pumps are used to pull the water out and treat it. These methods are very expensive. The Environmental Protection Agency estimates that it would cost *\$2.5 billion* to clean up all TCE-contaminated sites in the U.S.! Also, the usual decontamination methods are very impactful on the environment. For example, digging up contaminated soil and moving it elsewhere, far away from people, may reduce human risk, but the environmental problem is not solved, it just moved to another place. There are, however, alternative methods for cleaning up pollutants. Mother nature has plenty of tricks up her sleeve, and scientists have learned how to tap into that power. **Bioremediation** is the use of living organisms to destroy pollutants. One type of bioremediation is known as **phytoremediation**, which takes advantage of a plant's natural ability to remove toxins from soil. That is right, some plants can clean up pollution!

Not all plants are equally skilled at taking up pollutants, and if there is too much of a polluting chemical, it can kill the plant [1]. To avoid this, scientists focused on using poplar trees because they grow quickly (three meters per year), have lots of long roots, and can slowly destroy TCE [2]. You could call poplars the superhero of all trees. Still, some polluted sites have TCE concentrations that are too high for even poplar trees to handle. But, with the help of a very special bacteria, the problem can be solved.

## BACTERIA HELP PLANTS CLEAN UP THE ENVIRONMENT

How can a tiny bacterium be of help to a big tree? Well, scientists discovered a very special type of bacteria known as an **endophyte**. An endophyte is a bacteria or fungus that lives *inside* a plant and forms a **symbiotic** relationship with that plant host. This concept is similar to the helpful bacteria you and I have in our guts. Using several different mechanisms, endophytes can help plants tolerate many kinds of stresses, like droughts and salty soils. Some endophytes can even provide their hosts with essential nutrients. In return, the plants offer the endophytes sugar they make from photosynthesis, and a safe place to live.

Bacterial endophytes can live in practically any part of the plant. Before moving into their new plant home, endophytes reside in the

soil and must work hard to find a plant to live in. They may sense compounds released by plant roots to guide their way to a potential host plant. Think about a police dog sniffing its way to a treat. Once they reach the plant, the endophytes enter it through cracks in the roots [3].

But what about our TCE problem? A special bacterial endophyte known as PDN3 was used because it came from poplar trees and could grow in super-high levels of TCE [4]. The bacteria PDN3 was tagged green (Figure 1) so scientists could use a special microscope to verify that the endophyte could enter the plant. The next step was to get the PDN3 into poplar trees. This was done by simply soaking some poplar sticks in a bucket of bacterial solution. Then the sticks were poked into the ground of a TCE-polluted site. Another group of sticks, called controls, were also stuck into the ground, but were not added to the bacterial solution. By monitoring the trees with PDN3 and the control trees, biologists could see if PDN3 helped the trees clean up the TCE pollution. Look at the photograph in Figure 2. Do you think the bacteria helped the trees?

### Figure 1

The green you see in the photographs are -tagged PDN3 bacteria at (A) 48 h after being added to the poplar branch, and (B) 1 week after being added. These images, taken using a special microscope that can detect green fluorescence, show that the endophyte can enter roots through the cracks where roots join each other and can begin to live within the poplar tree [1].

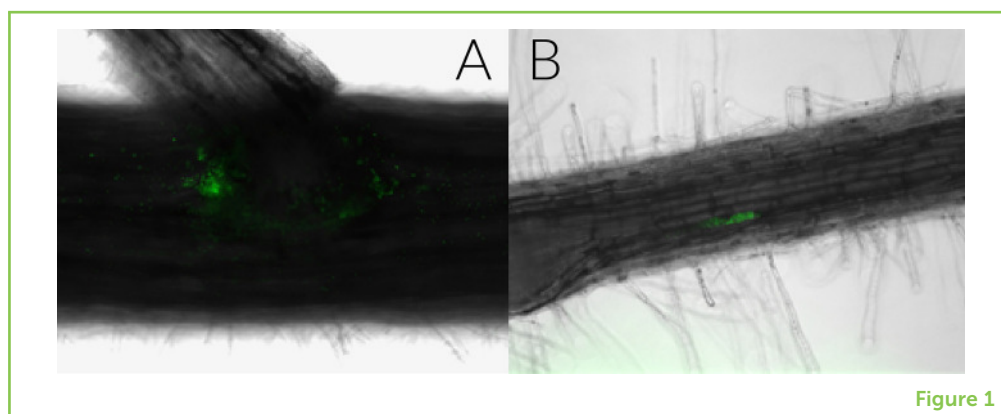


Figure 1

### Figure 2

In this photograph, you can see how much larger and healthier the trees with PDN3 looked, after just 1 year of growth. The control and PDN3-containing trees were planted in alternating rows. These results also demonstrated that the endophytes did not move from one tree to another, because the control trees did not benefit from the endophytes.

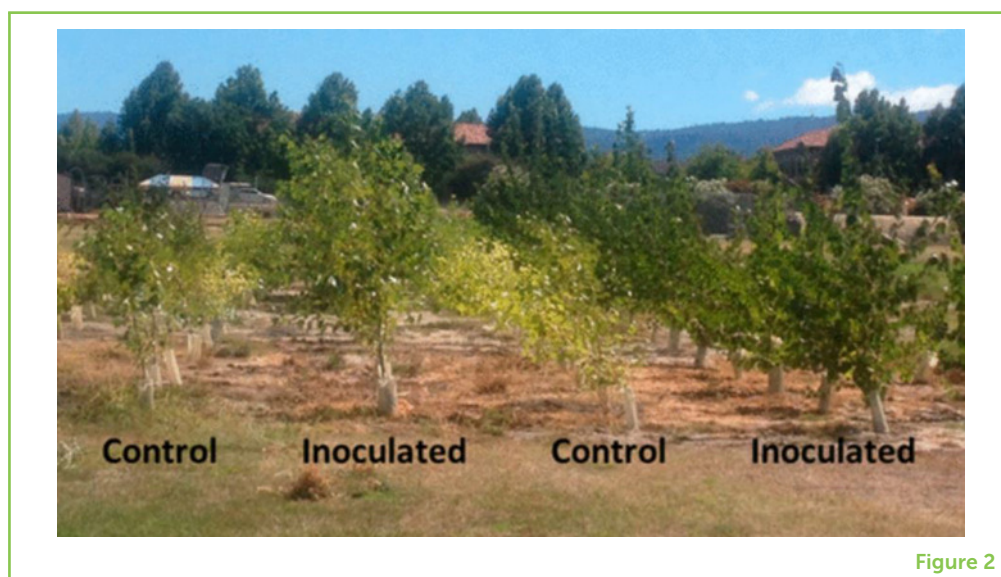


Figure 2

## WHAT IS ENDOPHYTE-ASSISTED PHYTOREMEDIATION?

We see that the trees with the added endophyte bacteria clearly look bigger and healthier than the control trees without PDN3. But did those bacteria help solve the TCE problem? Scientists collected several lines of evidence showing that the bacteria-tree partnership worked. The groundwater downstream of the trees had almost no trace of TCE 3 years after the trees were planted. So, the trees took up the polluted water but they did not look sick like the control trees did. The scientists checked inside the trees and learned that the trees given PDN3 had less TCE in their tissues than the control poplars had. This means that TCE taken up by the trees was destroyed inside the trees with the help of their endophyte friend. When TCE is broken down, chloride is released (since TCE is tri-*chloro*-ethylene). Sure enough, there was lots of extra chloride in the soil around the PDN3 trees. In other words, the endophytes helped the poplars reach more TCE and break it down. This process, in which plants and endophytes work together for restorative purposes, is called **endophyte-assisted phytoremediation**.

### ENDOPHYTE-ASSISTED PHYTOREMEDIATION

The use of endophytes to help plants remove pollutants.

Now we know the bacterial endophyte helped the poplar trees fix the TCE pollution problem, but scientists still wonder exactly how it works. Since the bacteria by itself can break down TCE really well, we know that the endophyte has special enzymes to do this. But endophytes also bring many benefits to their host plants, all of which may contribute to better removal of pollution. For example, endophytes such as PDN3 can make plant hormones that tell the plants to make more roots. The roots start growing quickly and can be longer and denser than they are in trees without PDN3. As a result, the PDN3-containing plants can pull more nutrients out of the ground. PDN3-containing poplar trees can also grow deeper and can possibly reach polluted regions that were previously unattainable.

## MOVING FORWARD

This discovery has the potential to change the way we clean up environmental pollution. Endophyte-assisted phytoremediation is a whole lot cheaper than the usual practices, it is very effective, and it is good for the environment. Likewise, it benefits wildlife as well as humans. But, because the discovery of endophyte-assisted phytoremediation is so new, the world still primarily uses the usual methods. The health of our planet depends on how we treat it, and it is our responsibility to clean up the mistakes of past generations. We must determine the future of our world by making the right decisions about how we manage our environment. By using the microorganisms

within plants and relying more on natural practices, we can make Earth a better home for all.

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## ORIGINAL SOURCE ARTICLE

Doty, S. L., Freeman, J. L., Cohu, C. M., Burken, J. G., Firrincieli, A., Simon, A., et al. 2017. Enhanced degradation of TCE on a Superfund site using endophyte-assisted poplar tree phytoremediation. *Environ. Sci. Technol.* 51:10050–8. doi: 10.1021/acs.est.7b01504

## REFERENCES

1. Doty, S. L., Freeman, J. L., Cohu, C. M., Burken, J. G., Firrincieli, A., Simon, A., et al. 2017. Enhanced degradation of TCE on a Superfund site using endophyte-assisted poplar tree phytoremediation. *Environ. Sci. Technol.* 51:10050–8. doi: 10.1021/acs.est.7b01504
2. Shang, T. Q., Doty, S. L., Wilson, A. M., Howald, W. N., and Gordon, M. P. 2001. Trichloroethylene oxidative metabolism in plants: the trichloroethanol pathway. *Photochemistry* 58:1055–65. doi: 10.1016/s0031-9422(01)00369-7
3. Kandel, S. L., Joubert, P. M., and Doty, S. L. 2017. Bacterial endophyte colonization and distribution within plants." *Microorganisms* 5:77. doi: 10.3390/microorganisms5040077
4. Kang, J. W., Khan, Z., and Doty, S. L. 2012. Biodegradation of TCE by an endophyte of hybrid poplar. *Appl. Environ. Microbiol.* 78:3504–7. doi: 10.1128/AEM.06852-11

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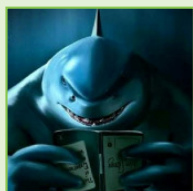
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**CONFLICT OF INTEREST:** JF is the Chief Scientific Officer of Intrinsyx Environmental which has a licensing agreement to use the TCE-degrading endophyte strain.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

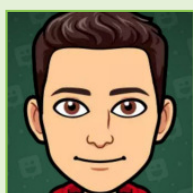
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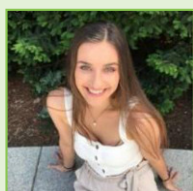
I am Aly, and I am interested in Biology, Paleontology, and reading about Animals and their behavior. I like Lizards, Sharks, and Bears.



### DARIO, AGE: 15

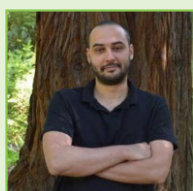
Hi my name Dario. I live in a small town in Austria with a big forest right aside my house. I often go for a walk with my two dogs. My favorite biological subjects are the adaptability and evolution of organisms over time.

## AUTHORS



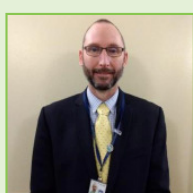
### LEILA ORDUKHANI

Leila Ordukhani is an undergraduate student at the University of Washington studying environmental science and terrestrial resource management. She grew up in California and has always been fascinated by science. At the UW, she has been able to further explore her interests in ecology, which is something she hopes to further pursue in graduate school in the years to come. Leila enjoys spending her weekends exploring the Seattle area with her friends.



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Andrea Firrincieli, Ph.D., is a postdoctoral research associate currently at the University of Bologna, Italy. Andrea is a microbiologist with experience in comparative genome analysis, plant-microbiome studies, and microbial ecology. His current Research Topics are the characterization *via* metagenome sequencing of microbial consortia exploitable for bioremediation.



### JOHN L. FREEMAN

John L. Freeman, Ph.D., is chief science officer of Intrinsyx Environmental at NASA-Ames Research Park, and researcher at NASA-Ames Earth Sciences Forestry Division Bio-Spheric Branch-SGE. His research includes microbial endophyte phytoremediation of petroleum hydrocarbons, chlorinated compounds, explosives, and tolerance mechanisms of metal/metalloid Hyperaccumulator and Salt/B

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### **SHARON L. DOTY**

Sharon L. Doty, Ph.D., has enjoyed studying the microorganisms associated with plants throughout her scientific career. Through her outreach and teaching, Doty emphasizes the ability of natural plant-microbe partnerships to address environmental challenges including agricultural chemical run-off, climate change, and pollution. When not in her lab, Sharon is often out where poplar trees thrive, alongside rivers in the Pacific Northwest. \*sldoty@uw.edu