

Phytoremediation

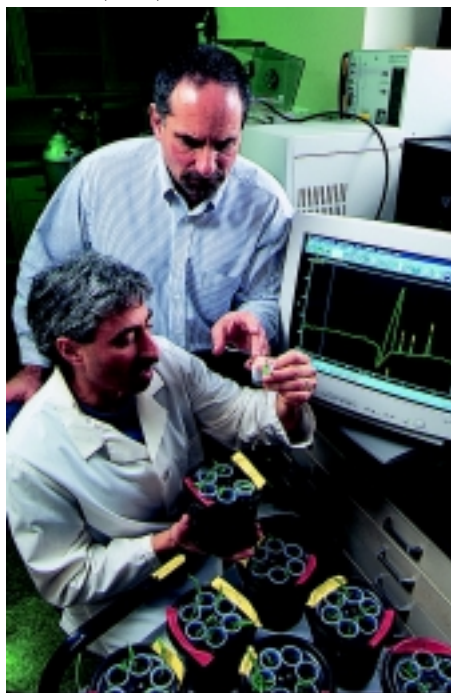
Using Plants To Clean Up Soils

When it comes to helping clean up soils contaminated with heavy and toxic metals, nature has ARS plant physiologist Leon V. Kochian to thank.

During 13 years of research at the U.S. Plant, Soil, and Nutrition Laboratory at Ithaca, New York, Kochian has become an authority on mechanisms used by certain plants to take up essential mineral nutrients and toxic heavy metals from soils. He has also characterized strategies some plants use to tolerate toxic soil environments.

Kochian is an international expert on plant responses to environmental stress, plant mineral nutrition, and use of plants

SCOTT BAUER (K8783-1)



Plant physiologist Leon Kochian (right) and Cornell University support scientist Jon Shaff analyze compounds released from sorghum roots.



Could this be a job for soil-cleaning superplants? The lack of vegetation in the barren area above is a result of the soil's high zinc content and low pH. This site in Palmerton, Pennsylvania, was contaminated by a zinc smeltery operated from 1890 to 1980.

to clean up or remediate soils contaminated with heavy metals and radioisotopes.

Besides providing important new information on how to use plants in this practical way, Kochian's research may also shed light on an important nutritional concern: how to prevent toxic metals from entering the food chain.

"One of the primary ways toxic heavy metals, such as cadmium, get in food is through plant uptake—the metal is taken up by the roots and deposited in edible portions," he says.

"Contaminated soils and waters pose major environmental, agricultural, and human health problems worldwide," says Kochian. "These problems may be partially solved by an emerging new technology—phytoremediation."

"Green" Technology: Simple Concept and Cost-Effective

Phytoremediation is the use of green plants to remove pollutants from the environment or render them harmless.

"Current engineering-based technologies used to clean up soils—like the removal of contaminated topsoil for storage in landfills—are very costly," Kochian says, "and dramatically disturb the landscape."

Kochian's cost-effective "green" technology uses plants to "vacuum" heavy metals from the soil through their roots. He says, "Certain plant species—known as metal hyperaccumulators—have the ability to extract elements from the soil and concentrate them in the easily harvested plant stems, shoots, and leaves. These plant tissues can be collected,



reduced in volume, and stored for later use.”

While acting as vacuum cleaners, the unique plants must be able to tolerate and survive high levels of heavy metals in soils—like zinc, cadmium, and nickel.

“Phytoremediation has been hampered historically by our inadequate understanding of transport and tolerance mechanisms,” says Kochian. To address this deficit, Kochian—working with ARS research associate Deborah L. Lethman, Cornell University postdoctoral associates Mitch Lasat and Paul B. Larsen, and graduate students Nicole S. Pence and Stephen D. Ebbs—has been studying a unique and promising metal hyperaccumulator. The plant is *Thlaspi caerulescens*, commonly known as alpine pennycress.

“*Thlaspi* is a small, weedy member of the broccoli and cabbage family,” Kochian says. “It thrives on soils having high levels of zinc and cadmium.”

His lab has been trying to discover the underlying mechanism that enables *T. caerulescens* to accumulate excessive amounts of heavy metals.

How Plants Clean Up

“Hyperaccumulators like *Thlaspi* are a marvelous model system for elucidating the fundamental mechanisms of—and ultimately the genes that control—metal hyperaccumulation,” says Kochian. “These plants possess genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plant.

“There are a number of sites in the plant that could be controlled by different genes contributing to the hyperaccumulation trait,” says Kochian. “These genes govern processes that can increase the solubility of metals in the soil surrounding the roots as well as the transport proteins that move metals into root cells. From there, the metals enter the plant’s vascular system for further transport to other parts of the plant and are ultimately deposited in leaf cells.”

Kochian’s team has gained insights into how, at the molecular level, *Thlaspi* accumulates these metals in its shoots at astoundingly high levels. “A typical plant may accumulate about 100 parts per million (ppm) zinc and 1 ppm cadmium. *Thlaspi* can accumulate up to 30,000 ppm zinc and 1,500 ppm cadmium in its shoots, while exhibiting few or no toxicity symptoms,” he says. “A normal plant can be poisoned with as little as 1,000 ppm of zinc or 20 to 50 ppm of cadmium in its shoots.”

The research also suggests an approach for economically recovering these metals. “Zinc and cadmium are metals that can be removed from contaminated soil by harvesting the plant’s shoots and extracting the metals from them,” he says.

After investigating the molecular physiology of zinc hyperaccumulation in *Thlaspi*, Kochian’s group found that several key sites for zinc transport were greatly stimulated in this plant. To get at the mechanism underlying the stimulation, they cloned a zinc transport gene—one of the first such accomplishments achieved with any plant. This breakthrough enabled the researchers to discover that zinc transport is regulated

KEITH WELLER (K6054-9)



Alpine pennycress doesn’t just thrive on soils contaminated with zinc and cadmium—it cleans them up by removing the excess metals.



Though the concept of phytoremediation is simple, progress is limited by the complexity of metal transport and tolerance mechanisms. Above, Cornell University research associate Miguel Pineros (left) and plant physiologist Leon Kochian study some of these mechanisms in corn.

differently in normal and hyperaccumulator plants.

“In normal plants, the activity of zinc transporter genes is regulated by the zinc levels in the plant,” he says. “In *Thlaspi*, however, these genes are maximally active at all times—independent of plant zinc levels—until you raise the tissue zinc levels to very high concentrations. This results in very high rates of zinc transport from the soil and movement of this metal to the leaves.”

It Even Works With Uranium

For soil contaminated with uranium, Kochian found that adding the organic acid citrate to soils greatly increases both the solubility of uranium and its bioavailability for plant uptake and translocation. Citrate does this by binding to insoluble uranium in the soil.

“With the citrate treatment, shoots of test plants increased their uranium con-

Today's "Phyto-miners" Rush to the Cry of "There's Metals in Them Thar Plants!"

Gold rush miners might have been better off using plants to find gold rather than panning streams for the precious metal.

Early prospectors in Europe used certain weeds as indicator plants that signaled the presence of metal ore. These weeds are the only plants that can thrive on soils with a high content of heavy metals. One such plant is alpine pennycress, *Thlaspi caerulescens*, a wild perennial herb found on zinc- and nickel-rich soils in many countries. This plant occurs in alpine areas of Central Europe as well as in our Rocky Mountains. Most varieties grow only 8 to 12 inches high and have small, white flowers.

In 1998, ARS agronomist Rufus L. Chaney and colleagues in ARS, at the University of Maryland, and in England patented a method to use such plants to “phyto-mine” nickel, cobalt, and other metals.



Agronomist Rufus Chaney examines the roots of a metal-accumulating *Thlaspi* plant in a growth chamber.

centration to over 2,000 ppm—100 times higher than the control plants,” he says. This demonstrates the possibility of using citrate—an inexpensive soil amendment—to help plants reduce uranium contamination.

Recently, Kochian, with colleagues Lasat and Ebbs, identified specific agronomic practices and plant species to remediate soils contaminated with radioactive cesium or cesium-137.

“Although the cause of cesium-137 contamination—aboveground nuclear testing—has been reduced, large land areas are still polluted with radiocesium,” Kochian says. “Cesium is a long-lived radioisotope with a half-life of 32.2 years. It contaminates soils at several U.S. Department of Energy (DOE) sites in the United States. Projected costs of cleaning up these soils is very high—over \$300 billion.”

Phytoremediation is an attractive

SCOTT BAUER (K8785-1)



Leon Kochian and ARS research associate Deborah Lethman study electrophoresis films to identify *Thlaspi caerulescens* genes responsible for heavy-metal transport.

alternative to current cleanup methods that are energy intensive and very expensive.

In initial lab and greenhouse studies, Kochian’s team showed that the primary limitation to removing cesium from soils with plants was its bioavailability. The form of the element made it unavailable to the plants for uptake.

In a series of soil extraction studies, Kochian’s team found the ammonium ion was most effective in dissolving cesium-137 in soils. This treatment increased the availability of cesium-137 for root uptake and significantly stimulated radioactive cesium accumulation in plant shoots.

Later, Kochian did field studies with six different plant species in collaboration with Mark Fuhrmann, a DOE scientist at Brookhaven National Laboratory in Upton, New York. They found significant variation in the effectiveness of

Chaney says biomining is the use of plants to mine valuable heavy-metal minerals from contaminated or mineralized soils, as opposed to decontaminating soils.

“The crops would be grown as hay. The plants would be cut and baled after they’d taken in enough minerals,” Chaney says. “Then they’d be burned and the ash sold as ore. Ashes of alpine pennycress grown on a high-zinc soil in Pennsylvania yielded 30 to 40 percent zinc—which is as high as high-grade ore. Electricity generated by the burning could partially offset biomining costs.”

USDA has signed a cooperative research and development agreement with Viridian Environmental, a technology company based in Houston, Texas. The CRADA involves Scott Angle at the University of Maryland; Alan J.M. Baker at the University of Sheffield, United Kingdom; plant breeder Yin-Ming Li with Viridian; and a cooperator at Oregon State University. Viridian is funding the CRADA’s phyto-mining research and development to the tune of \$1 million over 5 years.

Chaney says that to make phyto-mining as well as phytoremediation worthwhile requires, at a minimum, a plant with very high annual intake of minerals, such as the high-cadmium-accumulating pennycress variety for which they have filed a patent application.

“Better still, the traits of plants like pennycress could be incorporated into a high-yielding commercial crop like canola grown for hay,” Chaney says.

His idea of the best hyperaccumulators? “They’d have all the characteristics of a hay crop: They should be tall, high yielding, fast growing, easy to harvest, and deep rooted. And they should hold onto their mineral-rich leaves so they can be harvested along with the plant stems.”—By **Don Comis**, ARS.

Rufus L. Chaney is at the USDA-ARS Environmental Chemistry Laboratory, Bldg. 007, 10300 Baltimore Ave., Beltsville, MD 20705-2350; phone (301) 504-8324, fax (301) 504-5048, e-mail rchaney@asrr.arsusda.gov.



Leon Kochian (left) and molecular biologist David Garvin check wheat plants for aluminum tolerance. Some wheat and corn plants can tolerate aluminum by excluding the metal from the root tip.

plant species for cleaning up contaminated sites.

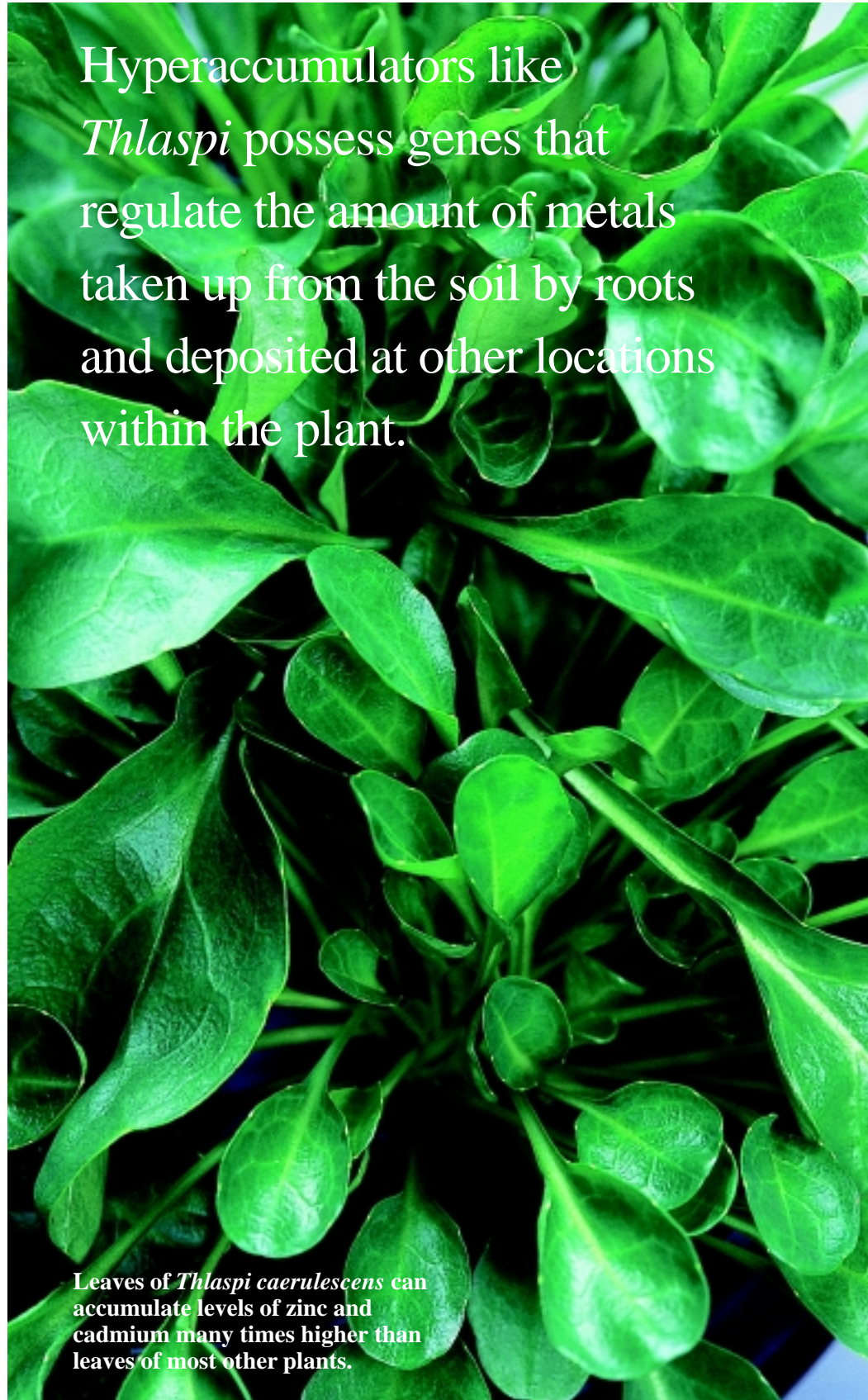
“One species, a pigweed called *Amaranthus retroflexus*, was up to 40 times more effective than others tested in removing radiocesium from soil. We were able to remove 3 percent of the total amount in just one 3-month growing season,” says Kochian. “With two or three yearly crops, the plant could clean up the contaminated site in less than 15 years.”

As a result of Kochian’s findings, DOE is performing pilot studies at Brookhaven using this technology.

Aluminum Hurts Crops Worldwide

Kochian’s lab is also working on finding ways to grow crops on marginal lands such as acid soils, where toxic levels of aluminum limit crop production. Aluminum is the third most abundant element in the Earth’s crust; it is a major component of clays in soil.

At neutral or alkaline pH values, aluminum is not a problem for plants.



Hyperaccumulators like *Thlaspi* possess genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plant.

Leaves of *Thlaspi caerulescens* can accumulate levels of zinc and cadmium many times higher than leaves of most other plants.

However, in acid soils a form of aluminum— Al^{+3} —is solubilized into a soil solution that is quite toxic to plant roots.

For years, scientists have been baffled by the causes of aluminum toxicity in plants.

“Aluminum toxicity limits crop production on acid soils, which cover well over half of the world’s 8 billion acres of otherwise arable land, including about 86 million acres in the United States,” Kochian says. “When soils become acid, the toxic aluminum damages plant root systems, which greatly reduces yields.”

Kochian’s research in collaboration with ARS plant molecular biologist David F. Garvin uses an interdisciplinary approach integrating molecular, genetic, and physiological research to provide insights into how particular genetic types of some plant species—including wheat, corn, and sorghum—can tolerate high levels of the metal in acid soils.

“We found that the root tip is the key site of injury, leading to inhibited root growth, a stunted root system, and reduced yields or crop failures from decreased uptake of water and nutrients,” Kochian says.

“Aluminum triggers the release of protective organic acids, specifically from the root tip into adjacent soil. When released, these acids form a complex with the toxic aluminum, preventing the metal’s entry into the root. Wheat and corn tolerate aluminum by excluding the metal from the root tip,” Kochian says.

Kochian is also conducting research on an aluminum tolerance mechanism in collaboration with plant molecular biologist Steve H. Howell of Boyce Thompson Institute at Cornell, using thale cress, *Arabidopsis thaliana*, a diminutive, weedy member of the mustard family.

He and colleagues have successfully identified *Arabidopsis* mutants that are aluminum tolerant. Kochian is studying differences between these mutants and a wild type of *Arabidopsis* to identify the molecular basis of tolerance.

The ultimate goal of this research is

Phytoremediation: Using Plants To Clean Up Soil



Leaves accumulate metals and are harvested to prevent soil recontamination.

Roots take up metals from contaminated soils and transport the metals to stems and leaves.

to isolate the genes conferring aluminum tolerance. It should then be possible to improve the tolerance of relatively aluminum-sensitive crop species, such as barley, or to further enhance the tolerance of existing aluminum-tolerant germplasm.

“One of the major goals for agricultural scientists for the immediate future is to increase food production to keep up with an ever-growing world population,” Kochian says. “As much of the world’s best agricultural land is already under cultivation or is being lost to industrialization, there is increasing pressure for farmers to cultivate marginal lands such as the huge expanses of acid soils that are not currently used for production.”

He continues, “Research aimed at producing crop genotypes that tolerate the suboptimal conditions of these marginal lands is one way global food production can be increased significantly. Being able to produce a wider range of crop species with increased aluminum tolerance will make a major contribution to these efforts to cultivate marginal,

stressed soil environments.”

Besides helping farmers who grow crops on acid soils, Kochian’s phytoremediation research findings are used by other scientists in government and academia and by environmental consultants, government, and industry groups complying with cleanup of contaminated sites.

For his landmark phytoremediation research, Kochian has received two awards: in 1999, the U.S. Department of Agriculture Secretary’s Honor Award for Environmental Protection and an award as ARS 1999 Outstanding Senior Scientist of the Year.—By **Hank Becker**, ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at <http://www.nps.ars.usda.gov/programs/cppvs.htm>.

Leon V. Kochian is with the USDA-ARS Plant, Soil, and Nutrition Laboratory, Cornell University, Tower Rd., Room 121, Ithaca, NY 14853-2901; phone (607) 255-2454, fax (607) 255-2459, e-mail lvk1@cornell.edu. ♦