

## "A plant genetically modified that accumulates Pb is especially promising for phytoremediation" (2003), by Carmina Gisbert et al.

In 2003, Carmina Gisbert and her research team produced a tobacco plant that could remove more lead from soil than normal tobacco plants. To do so, they inserted a gene from wheat plants that produces phytochelatin synthase into a shrub tobacco plant (*Nicotiana glauca*) to increase *N. glauca*'s absorption and tolerance of toxic metals, particularly lead. Gisbert and her team aimed to genetically modify a plant so that it could be used for phytoremediation—using plants to remove toxic substances from the soil. Scientists have identified phytoremediation as an effective and efficient process to improve human health and reproductive health in contaminated areas. Metals like mercury and lead can cause birth defects during human development like cognitive impairment, cerebral palsy, deafness, tremors, and blindness.

The research team from Valencia, Spain, included Roc Ros, Antonio De Haro, David J. Walker, M. Pilar Bernal, Ramón Serrano, and Juan Navarro-Aviñó. These researchers were located at the Consejo Superior de Investigaciones Científicas (Spanish National Research Council) and the University of Valencia in Valencia, Spain. Carmina and her colleagues published the results of their experiment in "A plant genetically modified that accumulates Pb is especially promising for phytoremediation" in 2003. The aim of the experiment was to identify and genetically modify a plant that grew rapidly, tolerated a wide range of environments, and accumulated high concentrations of toxic metals such as lead (Pb) and cadmium (Cd) from the soil in which it grew. In this experiment, the researchers showed that *N. glauca*, genetically modified to produce phytochelatin synthase, accumulated more lead in its roots, and also was more tolerant to cadmium, than non-modified *N. glauca*.

The first step of the experiment was to select a plant species that could tolerate and accumulate toxic metals to remove them from the soil. Phytoremediators—plants that can absorb toxic metals—grow slowly and produce small amounts of leaves and roots (plant biomass). According to the researchers, an ideal phytoremediator should grow quickly, produce a lot of biomass, accumulate and tolerate metals, and it must produce a high amount of unpalatable leaves to deter herbivores from eating them. That trait would prevent heavy metal transfer from animals to humans.

Gisbert and her colleagues conducted their research on a soil sample that was highly contaminated by hazardous industrial waste collected from the La Union mining site in the province of Murcia, in the southeastern part of Spain. They collected plant species from this contaminated site, and they selected some for further studies. Gisbert and her team focused on *N. glauca* and classified it as a hyperaccumulator, meaning that it could accumulate high levels of toxic metals in its biomass. Grown in regions that include Europe, Australia, and South and North Americas, the researchers noted that *N. glauca* had possible commercial and ornamental value as a phytoremediator.

In the next step of the experiment, the researchers used molecular biology techniques to insert the wheat gene TaPCS1, which produced phytochelate synthase, into *N. glauca* plants. Phytochelate synthase is an enzyme that binds with metal elements, and it is found in a variety of plant species. In 1999, a group of researchers had genetically inserted a phytochelate synthase gene from wheat (TaPCS1 gene) into yeast and showed that it caused yeast to accumulate cadmium. Gisbert and her colleagues inserted the wheat gene into *N. glauca* plants. The team infected *N. glauca* leaves with an *Agrobacterium tumefaciens* strain that contained DNA for phytochelate synthase. *Agrobacterium tumefaciens* is a species of bacteria that can transfer DNA between itself and plants. The scientists then isolated cells from the infected *N. glauca* leaves, grew the tissue in a variety of mediums and

conditions, and used offspring from that plant to test whether the TaPCS1 gene was successfully inserted into the tobacco plant, and whether or not it was producing the enzyme phytochelatase synthase.

In the final step of the experiment, the team conducted two sets of experiments to test the modified plants' capacity to absorb, accumulate, and tolerate toxic metals. The team tested the modified and non-modified (wild type) seedlings on nutrient plates, some of which were contaminated with lead and cadmium compounds, for nine days. The team also planted 10-day-old (mature) modified and wild type plants in a 50 percent dilution of contaminated soil for six weeks. They collected highly contaminated soil samples for analysis from Valencia's metropolitan areas and from one of the most contaminated sites, an old lead and zinc mine site at La Union. The soil sample contained a total concentration of 31,000 to 25,000 parts per million and more than 10,000 parts per million of lead and zinc respectively, which greatly exceeded the maximum permitted levels for agricultural soils in Europe. In both experiments, researchers collected the plants at the end of the time period, divided them into the roots and the leaves plus stem (shoots), and measured their weights. After eliminating the soil, and washing the material, they dried and analyzed the roots and shoots for heavy metals using atomic absorption spectrometry, a technique used to quantify the amounts of lead and cadmium in the plants. They found that the genetically modified plants absorbed about twice as much lead compared to non-modified plants.

## Sources

1. Clemens, Stephan, Eugene J. Kim, Dieter Neumann, and Julian I. Schroeder. "Tolerance to toxic metals by a gene family of phytochelatase synthases from plants and yeast." *The EMBO Journal* 18 (1999): 3325-33. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1171413/> (Accessed December 26, 2014).
2. Cobbett, Christopher. "Phytochelatins and their roles in heavy metal detoxification." *Plant Physiology* 123 (2000): 825-32. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1539264/pdf/hw0825.pdf> (Accessed December 26, 2014).
3. Eapen, Susan, and S. F. D'Souza. "Prospects of genetic engineering of plants for phytoremediation of toxic metals." *Biotechnology Advances* 23 (2005): 97-114.
4. Gisbert, Carmina, Roc Ros, Antonio De Haro, David J. Walker, M. Pilar Bernal, Ramón Serrano, and Juan Navarro-Aviñó. "A plant genetically modified that accumulates Pb is especially promising for phytoremediation." *Biochemical and Biophysical Research Communications* 303 (2003): 440-5.
5. Kotrba, Pavel, Jitka Najmanova, Tomas Macek, Tomas Ruml, and Martina Mackova. "Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution." *Biotechnology Advances* 27 (2009): 799-810.
6. Krämer, Ute. "Phytoremediation: Novel approaches to cleaning up polluted soils." *Current Opinion in Biotechnology* 16 (2005): 133-41.
7. Martínez, Mar, Pilar Bernal, Concepción Almela, Dinoraz Vélez, Pilar García-Agustín, Ramón Serrano, and Juan Navarro-Aviñó. "An engineered plant that accumulates higher levels of heavy metals than *Thlaspi aerulescens*, with yields of 100 times more biomass in mine soils." *Chemosphere* 64 (2006): 478-85.
8. O'Callaghan, Maureen, Travis R. Glare, Elisabeth P.J. Burgess, and Louise A. Malone. "Effects of plants genetically modified for insect resistance on nontarget organisms." *Annual Review of Entomology* 50 (2005): 271-92.
9. Raskin, Ilya, Robert D Smith, and David E Salt. "Phytoremediation of metals: Using plants to remove pollutants from the environment." *Current Opinion in Biotechnology* 8 (1997): 221-6.
10. Reeves, Roger D., and Robert Richard Brooks. "Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe." *Environmental Pollution Series A, Ecological and Biological* 31 (1983): 277-85.
11. Van Aken, Benoit. "Transgenic plants for phytoremediation: Helping nature to clean up environmental pollution." *Trends in Biotechnology* 26 (2008): 225-7.