



HEAVY METAL TOLERANT TRANSGENIC PLANTS

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Heavy metals such as cadmium, chromium, copper, lead, arsenic, nickel, and zinc contaminate urban soils at many sites throughout the world. Nations that continue to use leaded gasoline find toxic levels of lead in agricultural areas, making it difficult to raise animals and crops. Other heavy metals accumulate in soil and water from mining operations, industrial manufacturing facilities, recycling plants, and solid waste disposal sites. Military munitions are also major worldwide sources of groundwater and soil heavy metal contaminants, which wind or rain can sometimes disperse great distances from their point of use or disposal.

Traditional methods of removing heavy metals from soil and water are expensive and laborious, and often disrupt the environment. Contaminated soils can be excavated from the site and placed in a sanitary landfill, at a cost of approximately \$2,000,000 per acre. Not only is this method of heavy metal removal expensive, it may risk the spread of contaminated soil during removal. Alternatively, heavy metals can be stabilized and somewhat detoxified *in situ* using chelators. An example would be the addition of phosphate to soils contaminated with lead, forming an insoluble pyromorphite compound that remains inert in the soil. The cost of this fixation method is about half that of excavation. Another alternative is the use of plants to remediate heavy metals from soils. Phytoremediation can cost less than a quarter of the price of removal or fixation; however, the process can take much longer to be effective. Excavation and fixation of contaminated soils both require six to nine months on average for completion; by comparison, phytoextraction can take between 18 and 60 months.

Studies by the U.S. Environmental Protection Agency and the U.S. Military investigated the feasibility of phytoremediation for heavy metals cleanup¹. Their research looked at four mechanisms of heavy metal uptake by plants: phytoextraction, phytovolatilization, phyto-stabilization, and rhizofiltration.

The process of phytoextraction uses plants to absorb, concentrate, and precipitate heavy metals from soil. The metals accumulate in plant tissues where they are permanently stored. Plants called hyperaccumulators are preferred because they take up 100 times the concentration of metals over other plants. The plants are then discarded or processed to reclaim the metals.

Phytovolatilization is used to extract volatile metals such as mercury and selenium from sludge and soils and release them through transpiration to the atmosphere as a detoxified vapor.

Phytostabilization is used in sludge, soils, and spoils matrices. Plants are used to stabilize the metals by reducing water and wind erosion. In addition, the mobility of the contaminants is reduced by either being concentrated in root tissue, adsorbed onto roots, or precipitated in the root zone. Secretions into the rhizosphere precipitate the metals and bind them to solid particles in the matrix. The plants also dehydrate the matrix reducing the bulk needed for disposal. This procedure works well for keeping arsenic, cadmium, and lead from leaving the contaminated matrix.

Water is cleared of heavy metal contaminants using rhizofiltration. Plants growing in an *ex situ* or *in situ* hydroponics system are used to absorb, concentrate, and precipitate the metals, which remain in the roots. This technique works best with water tolerant plants having fibrous root systems. Cadmium and lead have been removed from contaminated water using this technique.

A recent summary of heavy metal phytoremediation concluded that the process could significantly decrease contamination over traditional methods, producing a 95% reduction in contaminated material disposed in landfills. However, the method has limitations. Natural plants have limited feasibility for remediation because of the toxicity of the metals to the plants and other inadequate growing conditions. In addition, because phytoremediation is confined to the area covered by the depths of the roots, the method is restricted to shallow



contamination sites and does not fully prevent the leeching of contaminants into groundwater. Also, complete remediation is a prolonged procedure because of the slow uptake of metals and small biomass of the plants. Finally, an increased threat of bioaccumulation occurs if the plants enter the food chain in the ecosystem.

Much of the earlier research on improving phytoremediation focused on finding hyperaccumulating plants. The research of Charles Rhyne and Sumita Ghosh at Jackson State University in Mississippi illustrates the criteria for plants regarded as cadmium and lead hyperaccumulators (refer to http://www-esd.lbl.gov/CEB/BEST/ann_rpt99/inter_9story.html). However, hyperaccumulators specific for particular compounds are difficult to find, and many of the plants take up the compounds under prescribed conditions that restrict their use in the field.

Youngsook Lee at the National Research Laboratory for Phytoremediation in Pohang, Korea, devised a strategy for reducing some of the limitations of heavy metal phytoremediation². Her team developed transgenic plants capable of tolerating high levels of accumulated cadmium and lead. These plants take up heavy metals more rapidly than traditional bioremediation plants, making them potential hyperaccumulators with application for phytoextraction and rhizofiltration in the field.

Lee observed that certain *Saccharomyces cerevisiae*, which possess the YCF1, or yeast cadmium factor 1 protein, is known to pump cadmium (Cd(II)) into vacuoles, and tested whether YCF1 would also confer resistance to lead (Pb(II)). Also known as vacuolar glutathione S-conjugate transporter, YCF1 belongs to the ATP-binding cassette superfamily^{2,3}. Lee's team confirmed that *YCF1* gene expression permitted *S. cerevisiae* to withstand the toxic effects of 3 mM lead (Pb II) and 0.1 mM cadmium (Cd II) concentrations in growth media. This protection against lead and cadmium toxicity was due to the uptake and storage of the heavy metals in yeast vacuoles. Next, Lee's group attempted to determine if *YCF1* expression in plants produced the same results.

Arabidopsis thaliana was investigated as a model for *YCF1* expression. First, the *YCF1* gene was created using RT-PCR from YCF1 expressing yeast. For expression in *A. thaliana*, Lee and colleagues subcloned the *YCF1* gene into two vectors—PBI121 and pCambia1302. To enhance expression in plants, the pCambia1302 vector was cloned with four copies of the CaMV 35S promoter. *Agrobacterium tumefaciens* was used for transformation of *A. thaliana*. Green fluorescent protein tagged to *YCF1*, used as an expression reporter, indicated the presence of YCF1 protein in the vacuolar as well as in the plasma membrane of the transformed *Arabidopsis* cells.

Lee and coworkers investigated the uptake and sequestering of lead and cadmium in the plants. Transformed *A. thaliana* was grown on gravel supplemented with half-concentration Murashige-Skoog agar medium containing 0.75 mM lead or 70 uM cadmium. After three weeks, the plant tissues were analyzed for metal uptake using atomic absorption spectroscopy. Lee's findings showed that the transgenic plants were as effective as naturally occurring hyperaccumulators. Although the transgenic plants accumulated less than two fold higher concentrations of Cd and Pb compared to wild type, this is likely much less than the hyperaccumulator plants (mentioned above).

Plants used for metal phytoremediation have few purposes after they have done their work, as the levels of metal taken up by the tissues may make them unsuitable for agricultural use, although they may have value if methods for inexpensively reclaiming the metals from the plant tissues are refined. Alternatively, heavy metal accumulating plants can be incinerated and the ashes disposed, which is much easier than excavating and disposing the contaminated soil. Although it is highly unlikely heavy metal accumulating plants will ever be used for food, production of non-toxic crops in heavy metal-contaminated areas may be developed from plants that exclude heavy metals, as recently described in *Plant Physiology* by Lee and colleagues⁵.



References

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