The Mess

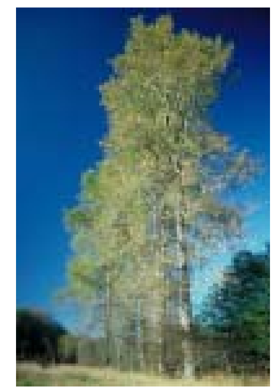
Over the last century, global industrialization, war, and natural processes have resulted in the release of large amounts of toxic compounds into the biosphere. Pollutants fall into two main classes: inorganic and organic. Inorganic pollutants comprise heavy metals such as cadmium, mercury, and lead; nonmetallic compounds like arsenic; and radioactive nuclear waste. Organic contaminants include petroleum hydrocarbons, solvents, phenolic compounds, explosives, fertilizers, herbicides, and pesticides.

Pollution is a huge global environmental problem. For example, 11.000 tons of mercury are released into the biosphere each year *(1)*. There are 12,000 contaminated sites listed in the United States, and 400,000 contaminated sites in Western Europe, with thousands of additional sites throughout the world *(2)*. Widespread contamination affects large areas in developing countries, where the pressure to use polluted land and water for food production is also very high. The world market for remediation was estimated to be between US$ 15-18 billion in 1998, and is expanding *(2)*.

Conventional Remediation Strategies

Conventional remediation for polluted sites typically involves the physical removal of contaminants, and their disposal in a designated site. Physical remediation strategies therefore do not eliminate the problem, they merely shift it. In addition, physical remediation strategies are also very expensive, disruptive to the environment, may temporarily increase exposure to chemicals, and often leave residual contamination.

PHYTOREMEDIATION



Phytoremediation, the use of plants to remove or degrade contamination from soils and surface waters, has been proposed as a cheap, sustainable, effective, and environmentally friendly alternative to conventional remediation technologies. Plants use solar energy (through photosynthesis) to extract chemicals from the soil and to deposit them in the above-ground part of their bodies, or to convert them to a less toxic form. These plants can then be harvested and treated, removing the pollutants.

An ideal phytoremediator would have: high tolerance to the pollutant; the ability to either degrade or concentrate the contaminant at high levels in the biomass; extensive root systems; the capacity to absorb large amounts of water from the soil; and fast growth rates and high levels of biomass.

Although several species can tolerate and grow in some contaminated sites, these species typically grow very slowly, produce very low levels of biomass, and are adapted to very specific environmental conditions. And trees- which have extensive root systems, high biomass, and low agricultural inputs requirements- tolerate pollutants poorly, and do not accumulate them. Conventional plants therefore fail to meet the requirements for successful phytoremediators.

Cleaning Up More Efficiently with Green, Biotech "Mops"

The remedial capacity of plants can be significantly improved by genetic manipulation and plant transformation technologies. The introduction of novel traits for the uptake and accumulation of pollutants into high biomass plants is proving a successful strategy for the development of improved phytoremediators. This Pocket K reviews some of the research efforts in this field, and highlights future challenges.

Cotton flowers

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Tel: +63-2 8450563
Fax: +63-2 8450606
E-mail: knowledge.center@isaaa.org

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Pocket K No. 25

BIOTECH PLANTS *for* BIOREMEDIATION

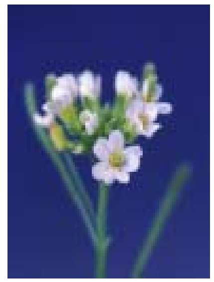
GLOBAL KNOWLEDGE CENTER ON CROP BIOTECHNOLOGY

Cadmium, Zinc, Lead, and Selenium

Toxic metals affect crop yields, soil biomass, and fertility, and accumulate in the food chain. Metal-tolerant species protect themselves from the toxicity of metal ions by binding metals ions with specific proteins that render them in a safer form. Three classes of proteins are important for metal detoxification in plants: metallothioneins, phytochelatins, and glutathione. The genes coding for these have been successfully used to improve phytoremediators through genetic engineering.

For example, shrub tobacco (*Nicotiana glauca*) transformed with the phytochelatin *TaPCS1* shows very high levels of accumulation of zinc, lead, cadmium, nickel, and boron, and produces high biomass *(3)*. In Arabidopsis, Indian mustard, and tobacco plants, improved metal tolerance was achieved through the over-expression of enzymes that induce the formation of phytochelatins (*4, 5,* and *6*).

Plants naturally tolerant to heavy metals have also been used as a source of genes for phytoremediation. Transgenic Arabidopsis plants expressing a selenocysteine methyltransferase (*SMTA*) gene from the selenium hyperaccumulator *Astralagus bisulcatus* contain eight times more selenium in their biomass when grown on selenite compared to non-transgenic controls. Comparison of gene expression profiles between *Arabidopsis thaliana* and the closely related species *A. hallerri*, which is tolerant to cadmium and hyperaccumulates zinc, is also helping identify major genes required for metal tolerance (*6*).



Arabidopsis inflorescence

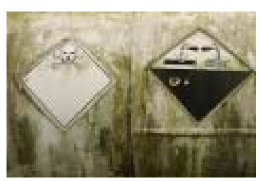
Herbicides

Mammalian P450 cytochrome genes have been used to confer herbicide resistance to crop plants, which can be used in herbicide rotation systems designed to delay the evolution of herbicide resistance in weeds, and to reduce the environmental load of agricultural chemicals (5, 6). Herbicide resistance is also provided by the over-expression of the maize glutathione S-transferase I (*GSTI*) gene (6).

Explosives

Millions of tons of explosives have been released into the environment, with the resulting pollution of vast expanses of land and water resources. RDX (Research Department Explosive) was the primary explosive used during World War II, and newer derivatives are extensively used to date. Explosives, and their degradation products, are extremely toxic and corrosive.

Tobacco plants engineered with the bacterial gene for a NADPH-dependent nitroreductase tolerate and degrade high levels of TNT (9), and Arabidopsis plants carrying the xplA gene from Rhodococcus bacteria are highly resistant to of RDX (6).



Landmines

Landmines affect millions of people, both combatants and civilians, in over 80 countries. Sixty to 70 million active landmines exist throughout the world, and these claim the lives and limbs of 50 people every day, and threaten the livelihood of many more by denying them access to humanitarian aid, agricultural land, and water resources. Efforts are underway to develop transgenic plants that can be used to warn civilians of the presence of landmines in a field (10). Arabidopsis whose roots change color when they come into contact with degradation products of landmines have been developed. Scientists are now working to allow the plant to transmit the signal to their leaves, to effect human-readable changes for a practical explosives detection system.

Mercury

Mercury is a highly toxic element found both naturally and as an introduced contaminant in the environment, and is a very serious global environmental problem. Organic mercury (organomercurials), the most toxic form to living organisms, is produced when bacteria in the water and soil convert elemental mercury into methylmercury. Methylmercury is easily absorbed and accumulates at high levels in the food chain. Mercury poisoning affects the immune system, damages the nervous system, and is harmful to developing fetuses.

Detoxification of organomercurials has been achieved in transgenic plants by transforming Arabidopsis, tobacco, poplar trees, Indian mustard, and eastern cotton wood with two bacterial genes, *merA* and *merB*. (*5, 6, 7*). The combined actions of *merA* and *merB* transform methylmercury to the volatile elemental form, which is 100 times less toxic, and is released by the plant to the atmosphere at non-toxic concentrations through transpiration.

Arsenic

Arsenic occurs naturally in rocks and soil, and is released into underground water. Consumption of contaminated drinking water leads to skin disorders, gangrene, and cancer of the kidneys and bladder. In addition, high levels of arsenic in agricultural land degrade soils, reduce crop yields, and introduce the pollutant to the food chain (*8*). Arsenic contamination threatens up to 40 million people in Bangladesh alone, a problem described by the World Health Organization (WHO) as "the largest poisoning of a population in history".

Scientists have engineered Arabidopsis plants with arsenic tolerance by introducing two bacterial genes: *arsC* and *ã-ECS*. *arsC* converts arsenate, the arsenic form absorbed by plants, to arsenite. Double transgenics are not only highly tolerant of arsenic, they also have improved cadmium tolerance, and a six-fold increase in the level of biomass compared to wild-type controls (*6*).

Prospects

Although the use of biotechnology to develop transgenic plants with improved potential for efficient, clean, cheap, and sustainable bioremediation technologies is very promising, several challenges remain.

Field of tobacco plants

- A better understanding of the molecular basis of the pathways involved in the degradation of pollutants is needed. Further analysis and discovery of genes suitable for phytoremediation is essential.
- Phytoremediation technologies are currently available for only a small subset of pollutants, and many sites are contaminated with several chemicals. Therefore, phytoremediators need to be engineered with multiple stacked genesin order to meet the requirements of specific sites.
- Phytoremediation technology is still at an early development stage, and field testing of transgenic plants for phytoremediation is very limited. Biosafety concerns need to be properly addressed, and strategies to prevent gene flow into wild species need to be developed.
- The true costs of benefits of phytoremediation with biotech plants must be determined.